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Editorial
The Bhutanese Journal of Agriculture welcomes the submission of research articles and short communications. Article may be submitted to *bja@moaf.gov.bt*. Please follow the journal format provided in the author guidelines, accessible from *www.bja.gov.bt* or *www.doa.gov.bt*. The online open access for the Bhutanese Journal of Agriculture is now available at *www.bja.gov.bt*.

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FOREWORD

The Department of Agriculture is delighted to bring out the fourth volume of the Bhutanese Journal of Agriculture (BJA). BJA is a print open access English language journal on agriculture and publishes research articles annually with the primary purpose of providing an additional mechanism to disseminate appropriate technologies, and knowledge and information in the agriculture sector.

Across cross-cutting themes like agronomy, postharvest, economics, agro-biodiversity, soil and water management, farming systems, pest and disease management and climate change, the journal covers research findings that are innovative and relevant to sustainable agriculture development in Bhutan.

It has been a difficult year with the Covid-19 pandemic affecting everyone across the board. The department has, in its own little measures, made significant reforms in its approach to agriculture development so as to help deliver immediate recourse to our farmers as well as the economy. At the same time, our efforts into empirical and objective research to help develop data-driven technology should continue unabated. This volume which we are able to release uninterrupted is thus a testament to our collective resolve in seeking new and relevant technologies and effectively communicating them through a peer-reviewed platform.

Our editorial team, comprising members entirely from within the Department of Agriculture, is pushing hard to conform to international standards. Concerted efforts are underway in continuously improving the quality of the journal and we are glad and hope that with every passing issue we enrich ourselves with experiences that enable us to set the bar ever higher.

I thank the authors and the reviewers alike for their contribution as well as the BJA Editorial Board for their added efforts in successfully taking out this edition. I wish everyone a resourceful reading.

Stay safe and best wishes.

Kinlay Tshering (Ms)

DIRECTOR

EDITORIAL

The COVID-19 pandemic has tested our resilience and taught us far-reaching lessons. The most pertinent ones concern those that test the reliability and elasticity of our agriculture and food systems, medical preparedness, supply chain management, and infrastructures for virtual communication and digital information exchange. The strengths of domestic food production and the consequences of heavy dependence on external food supply have become all the more apparent. Once we find ourselves on the other side of the pandemic divide, the ‘washing-hands’ may gradually and as well shift to ‘dirtying-hands’ with local food production taking centre-stage to preempt crises in food and nutrition.

It is hoped that against such emerging contexts the technologies and knowledge generated and presented in this volume of the Bhutanese Journal of Agriculture (BJA) will only build on and strengthen our agriculture and food systems, and serve as enriching literature for future endeavours in this sector. There are a set of 12 interesting articles that capture varied disciplines from crop germplasm evaluation and innovation testing to new insights into postharvest processing. Fresh understandings into aspects on rice, quinoa, bean, chirayita, kiwifruit, potato, groundnut, mango, pear and apples are presented, including assessment of performance of farmers’ groups and cooperatives. The volume also covers work on biochar and evaluation of local yeast strains in winery.

We are pleased to report that the quality of articles in this journal is improving. The percentile of acceptance of articles has risen to around 50 now. We are grateful to all authors, reviewers, facilitators and the journal editorial board for their concerted effort and diligence in ensuring this volume a success. On behalf of the journal board, I extend our gratitude to all contributing institutions including the Agriculture Research and Development Centres at Wengkhar, Samtenling and Bajo, the National Post Harvest Center (Paro), the National Centre for Organic Agriculture (Yusipang), the College of Natural Resources (Lobesa), the Regional Agriculture and Marketing Cooperatives Office (Mongar), and above all the Department of Agriculture for providing the resources for these research and for supporting the authors and collaborators alike. We rededicate ourselves to generating evidence-based knowledge and credible technologies to help take forward sustainable agriculture development in the country.

We hope our readerships find the articles useful and interesting. Thank you to all our readers and users.

Tashi Delek!

(Yadunath Bajgai, PhD)

Editor-in-Chief

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Evaluation of New Mango Varieties in Samtenling, Sarpang

Tshering Yangden[a], Ugyen Tshering[a], Kezang Euden[a], Sangay Dorji[a], Ngawang[a], Gangaram Ghalley[a]

ABSTRACT

Evaluation of four varieties of mango was carried out at Agriculture Research and Development Centre, Samtenling during 2016-2018 to characterise the varieties with basic morphological traits and to assess their performance under sub-tropical agro-ecological zone. The varieties evaluated were Chinwang, Irwin, Duncan and Tommy Atkin with Amrapali as a check. Description of morphological traits is based on IBPGR mango descriptors. For performance evaluation, yield data and fruit quality analysis were recorded. Significant differences were observed in the quality analysis of fruits from all the varieties tested against the check variety. Maximum fruit weight was recorded in Chinwang variety with the mean weight of 893 gram and minimum was recorded in Amrapali (194 gram). The TSS result from all the four varieties did not show any significant difference (P value 0.0998) with the mean TSS of 13.2. Average yield per tree was also recorded in Chinwang (81kg/ tree) followed by Tommy Atkin (79 kg/tree), Duncan (69 kg/tree) and Irwin (33 kg/tree and lowest yield in the check variety Amrapali (19 kg/tree). Although all varieties were short stature in growth habit, Chinwang was found to be taller (5.2 m) than other varieties (Amrapali (4.7 m), Duncan (4 m), Irwin (3.9 m) and Tommy Atkin (4.0 m)). All four varieties have the potential for further promotion. However, continued multi-location evaluation will determine their performance under different climate and soil conditions.

Keywords: *Growth habit; Physiological characters; Potential, Yield*

1. Introduction

Mango (*Mangifera indica* L.) is also known as the king of fruits and is one of the most important fruit crops from the family anacardiaceae. It is highly valued and can grow from sea level to altitudes up to 1100 m (Chadha, 2014). It is the national fruit of India, Bangladesh and the Philippines. It is said to be originated from the Indo-Burma region. Domestication of mango in India dates back to 4000 B.C. (Mehta, 2017). The genus Mangifera contains almost 49 species which are mostly distributed in its centre of origin. It is believed to have reached China in the 7th century; East Africa in the 10th century and to the Philippines at the beginning of the 15th Century. Later from the South and Southeast Asia, it spread to many tropical and subtropical regions of the world. Now it is cultivated in over 89 countries (Yadav & Singh, 2017). The world area under mango cultivation is almost 6.2 million ha with a total production of 55 million metric tonnes. The

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major mango producing countries are India, China, Thailand, Indonesia, Mexico, Pakistan, Brazil, Bangladesh and Egypt (FAO, 2019). FAOSTAT data for mango include mangosteen and guava but some research states that in most countries, mango accounts for more than 99% of the data (Requena, 2015).

In Bhutan, mango cultivation is confined to wet tropical to dry subtropical regions covering a total area of 402.55 ha with a production of 530 MT (DoA, 2017). The varieties available are Bajo Amchukuli 1, Bajo Amchukuli 2, Bajo Amchukuli 3, and Chausa (DoA, 2020). Globally, mango is one of the most preferred fruits and consumed all across at a rate exceeding 5 kg per capita with a trade value of US$ 60 billion (Requena, 2015). Being a potential crop worldwide, the Department of Agriculture has identified it as one of the prioritized fruit crops with a mandate to increase its production and emphasize research into it.

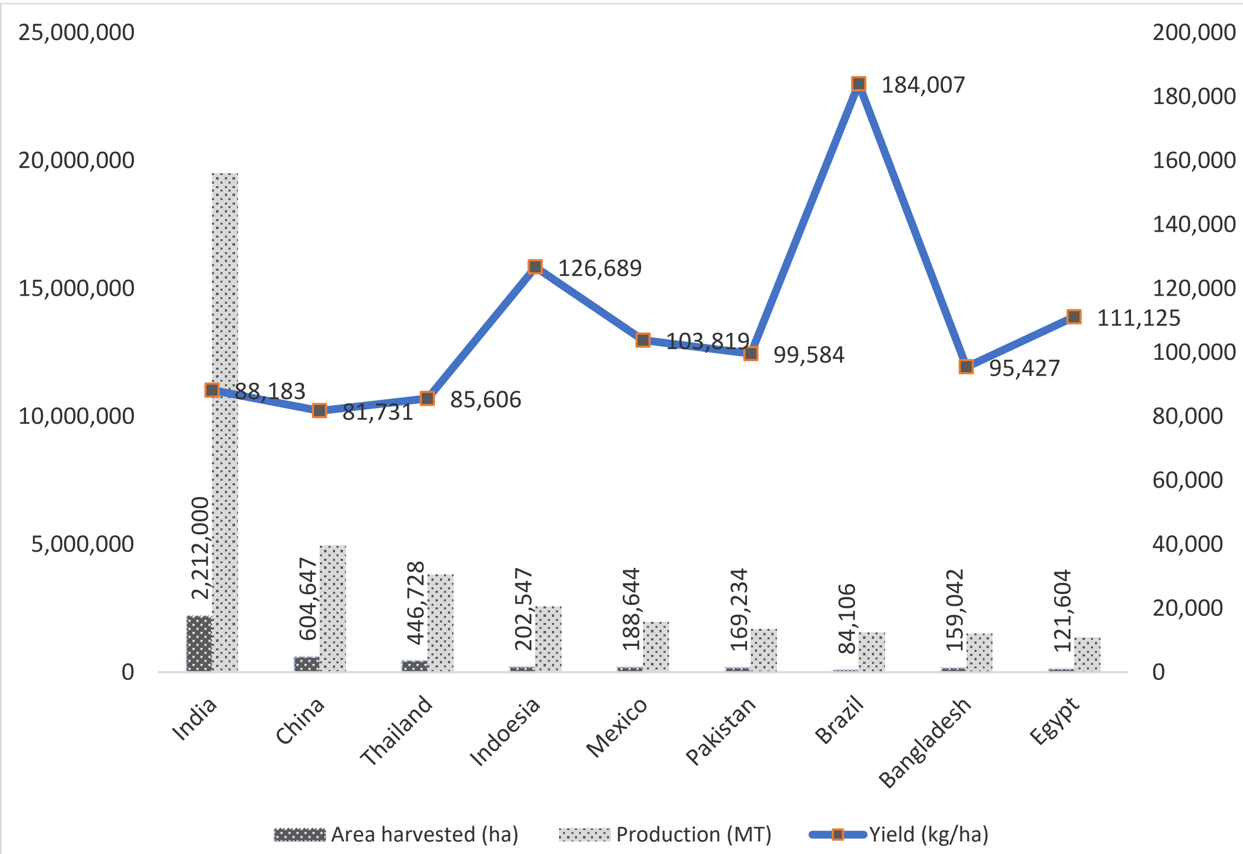

Figure 1. Top mango producing countries (Data source: FAO, 2017).

One of the areas of research is to introduce and evaluate the performance under different agro-ecological zones in the country. Very few known elite varieties are cultivated presently. Few varieties that are released till date are all sourced from India with an alternate bearing habit which is a major drawback in mango production. Moreover, the existing varieties of mango date back to

2002 and 2010 and there is no new variety released thereafter. Therefore, mango germplasm evaluation was carried out at the Agriculture Research and Development Centre (ARDC), Samtenling, on four varieties introduced from Thailand starting from 2004. The evaluation was conducted to determine their performance, assess quality characters of the varieties and to characterise them according to basic morphological traits. The varieties evaluated were Chinwang, Irwin, Duncan and Tommy Atkin.

2. Materials and Method

2.1. Location and experiment details

Evaluation trial was conducted at ARDC Samtenling situated in the wet sub-tropical agro-ecological zone (Latitude of 26.9^{o}N, Longitude 90^{o}E, altitude: 375 masl). It experiences an annual rainfall of 1000-1500 mm with an annual average maximum temperature of 27.6^{o}C and annual average minimum temperature of 20.6 ^{o}C (NCHM, 2017). The scion woods of the four varieties were collected from ARDC Bajo, grafted and systematic plantation was done in 2008. They were tested against the check variety Amrapali. Planting distance of 7m (PP x RR)* was maintained. Recommended fertilizer dose at 250:160:600 g/plant/year (N:P:K) were applied to all the trees conforming to the nutrient content of the soil obtained from soil test results (Table 1). All the treatments were provided with uniform orchard management practices.

Table 1. Soil test result of the trial site.

	N (%)	Texture: Sandy Loam	pH	P (kg)	K (%)
Top soil	0.06 (vL)	Sandy loam	4.15	100.1 (vH)	15.58 (vL)
Sub soil	0.10 (L)	Sandy loam	4.27	114.41(vH)	0.05 (vL)

- vL: very low
- L: low
- vH: very high

(PPxRR)*: plant to plant & row to row distance

2.2. Variety details

The experiment was conducted with a treatment of five varieties (Chinwang, Irwin, Duncan and Tommy Atkin) including Amrapali as check variety. The three varieties viz. Irwin, Tommy Atkin and Duncan are originally from Florida. Irwin cultivar is a Florida selection developed from the parent materials Lippens x Hadens (Olano, Schnell, Quintanilia, & Campbell, 2005). Tommy Atkin developed from seed was originally grown in Broward County in Florida from where one Mr. T. H. Atkins bought and grafted the variety (Campbell, 1973). Duncan is the hybrid between Edward x Pico No. 18 which was bred by David Sturrock from West Palm Beach County of Florida (Sturrock, 1970). Amrapali is a dwarf hybrid from a cross between Dashehari x Neelum developed in India (Das, 2013).

2.3. Fruit quality analysis

Fruit samples were collected from three numbers of trees. Ten numbers of fruits were collected at random and analysed in the laboratory for various quality traits like fruit weight (g), Stone weight (g), pulp weight (g), fruit length (cm), Fruit diameter (cm), pulp percentage (%) and TSS (%). Pulp percentage was calculated by the weight of pulp with peel divided by the total weight of the fruits multiplied by 100.

2.4. Morphological characterisation of fruits and trees

Morphological characterisation of fruits and trees were carried out following the standard guidelines of "descriptors for mango (*Mangifera indica*)" developed by the International Plant Genetic Resources Institute (IPGRI) (IPGRI, 2006). The height of trees was measured from ground level to the top of the tree. Trunk circumference was measured at 50 cm above the ground in mature trees. Similarly, crown diameters were measured as the mean diameter using two directions (north-south and east-west). Morphological traits on fruits assessed were fruit shape, shape of fruit apex, fruit ground colour, fruit flush colour, depth of fruit stalk cavity, fruit neck prominence, slope of ventral shoulder, fruit beak type, fruit sinus type and pulp colour of ripe fruits. Observation on the initiation of flowering, 50% flowering, full bloom and days to fruit ripening were also recorded.

2.5. Yield data collection

The yield of the tree (kg/tree) and the number of fruits per tree were recorded.

2.6. Statistical analyses

All the observations were recorded from three numbers from each of the varieties. Data were recorded in Microsoft Excel worksheet and were analysed using statistical tool STAR 2.0.1and were subjected to one-way analysis of variance (ANOVA) at 5 % level of significance.

3. Results and Discussion

3.1. Fruit quality analysis

Significant differences were observed in almost all the varieties tested against the check variety (Table 2). Highest fruit weight was observed in Chinwang and minimum was recorded in Amrapali. The mean fruit weights of the other three varieties (Irwin, Tommy Atkin and Duncan) were similar but better than Amrapali variety. The stone weight of Chinwang was significantly higher than in other varieties. Significant variations were also found in the pulp weight of Chinwang against all the varieties. Pulp percentage of Chinwang is significantly different from Tommy Atkin and Amrapali but at par with Duncan and Irwin. Similarly, pulp percentage of Tommy Atkin is significantly different from Duncan and Chinwang but at par with Irwin and Amrapali. The highest fruit height was noticed in Chinwang which is significantly different from the other four varieties. No significant differences were found in the fruit heights of Duncan. Irwin and Tommy Atkin. The check variety with an average fruit height of 9.2 cm is at par with Tommy Atkin but significantly different from the other three varieties. Similarly, a significant difference was also observed in the fruit diameter (*P= 0.025*). The TSS result from all the four varieties did not show any significant difference. The mean TSS observed was 13.2 at significant test level of ($\alpha = 0.01$).

Table 2. Comparison of means on quality properties of the varieties.

Variety	Mean fruit weight (g)	Stone weight (g)	Pulp weight with peel(g)	Pulp percentage (%)	Fruit Height (cm)	Fruit diameter (cm)	TSS (%)
Chinwang	892.86a	61.73a	831.14a	92.99a	17.86a	10.25a	13.59
Duncan	425.97b	35.26b	390.60b	91.73a	11.50b	8.35abc	12.23
Irwin	418.30b	36.35b	381.95b	91.21ab	11.59b	8.15 bc	11.73
Tommy Atkin	401.35b	42.54b	358.80b	87.99 bc	10.27bc	8.49ab	13.33
Amrapali	194.07c	28.15c	165.93c	85.36c	9.23c	6.47c	14.94
Mean	466.51	40.81	425.68	89.86	12.09	8.34	13.16
P-value	0.0006	0.0125	0.005	0.0038	0.000	0.0253	0.0998
CV (%)	23.45	21.27	23.8	1.94	7.89	12.41	9.85

**Means followed by the same letter in the column are not significant (P<0.01)*

For any crop improvement program, quality characters associated with the variety is the most important tool for further selection and breeding of the crop (Desai, Musmade, Ranpise, & Chaudhari, 1994). Fruit weight is one of the important traits that contribute to the yield of fruit plants. Study has shown that there is a strong inherent correlation between the traits like fruit weight, plant height and percent perfect flower on fruit yield in mango (Majumder, Hassan, Rahim, & Kabir, 2012). Studies have further affirmed that fruit weight, fruit diameter, stone size and titrable acidity are major components of mango fruit yield which need to be considered in mango improvement programs (Lal et al., 2017). In hybridization of the crops heritability of traits are important attributes. Heritability studies in mango have shown that there is a heritability of >0.9 for traits like fruit weight, fruit length, width, thickness and TSS (Dinesh, Vasugi, & Venugopal, 2010). One of the consumer preferences is fruit size and taste of the fruits. Studies have also found out that one of the factors that determine the consumer preferences over the variety of the fruits are taste, quality and pulp (Shukla, Chaudhari, & Joshi, 2014). With regular bearing habit and other superior quality, the varieties have the potential for cultivation in increasing production and can be used in crop improvement through breeding.

3.2. Yield

Among the five varieties, the maximum average yield was recorded in Chinwang (Table 3) followed by Tommy Atkin, Duncan and Irwin. The lowest yield is noticed in the check variety Amrapali. However, the yield does not show a significant difference among the three varieties (Chinwang, Tommy Atkin and Duncan), but they are significantly different from Amrapali and Irwin. There is no significant difference in the number of fruits for all the varieties. During the observation on the varieties, it was noticed that all the four varieties were regular bearing while Amrapali is an alternate bearing. Hassan (as cited in Hafiz, Hossain, & Karim, 2018) refers to the regular bearing habit in Amrapali.

Table 3. Comparison of mean yield.

Varieties	**Mean yield (kg per tree)**	**Avg. no. of fruits per tree**
Chinwang	81.35a	121.33
Duncan	69.03a	176.00
Irwin	32.76b	84.00
Tommy Atkin	79.49a	163.33
Amrapali	19.07b	134.33
Mean	57.34	135.80
CV (%)	24.39	27.44
P-value	0.0014	0.972

** Means followed by the same letter in the column are not significant (P<0.01)*

3.3. Observation of quantitative vegetative characters of trees

It is observed that variety Chinwang is vigorous in growth habit (Table 4) followed by Amrapali, Duncan Irwin and Tommy Atkin. Therefore, all the varieties can be considered as short stature plants. The highest spread of tree was noticed in the variety Amrapali (6.06 cm) and it is significantly different from all the four varieties. The average girth of the trunk also varied significantly (*P=0.0022*) with the highest girth recorded in Amrapali (72.80 cm) and least in Duncan (49.20 cm).

Table 4. Mean comparison of vegetative growth of the tree.

Variety	**Canopy height (m)**	**Spread of trees (cm)**	**Girth of trunk (cm)**
Chinwang	5.15a	4.93b	66.50ab
Duncan	4.00b	4.24c	49.20c
Irwin	3.98b	3.79c	53.00c
Tommy Atkin	4.02b	3.91c	57.14bc
Amrapali	4.72a	6.06a	72.80a
Mean	4.38	4.59	59.73
CV (%)	11.34	11.19	14.03

P-value	0.0044	0	0.0022

Plants with heights less than six metres are described as short stature as per the IPBGR descriptor for mango (IPGRI, 2006). These four varieties can be suitable for high-density plantation and intensive farming. High-density planting has been tried in Tommy Atkin varieties at various spacing of 8 x 5 m, 7 x 4 m, 6 x 3 m, 5 x 2 m and 4 x 2 m. The reduction in yield and number of fruits per tree was noticed but the yield per unit area increased by 30% (Sousa et al., 2011). The reduction in the canopy size, girth of trunk and fruit height have been reported by (Gaikwad, Chalak, & Kamble, 2017) as well which provides evidence on the requirement of short stature plant for high-density plantation.

3.4. Morphological characterisation of fruits

Morphological characterisation fruit shape, shape of fruit apex, fruit ground colour, fruit flesh, depth of fruit stalk cavity, fruit neck prominence, slope of the ventral shoulder, fruit beak type, fruit sinus type and pulp colour of ripe fruits for all the five varieties were done following the guidelines of "mango descriptor" developed by IPGRI. The fruits of Chinwang, Irwin and Amrapali are oblong. Tommy Atkin possesses roundish fruit shape with round fruit apex. Similarly, Duncan is obovoid in shape. The ground colour of Chinwang and Amrapali is green with a red blush on Chinwang fruits. The external colour of Irwin and Tommy Atkin is excellent with red ground colour while Duncan fruit turns yellow on ripening with no blush. Acute fruit apex is noticed in Chinwang and Tommy Atkin, obtuse in Duncan and Amrapali, and round in Tommy Atkin. Chinwang, Irwin, Tommy Atkin and Amrapali have shallow fruit sinus while it is absent in Duncan.

All the four varieties have a medium depth of fruit stalk cavity except in Amrapali which possess a shallow depth of fruit stalk cavity. All the varieties have perceptible fruit beak. The pulp colour is golden yellow in Chinwang, orange in Irwin, light yellow in Duncan and Tommy Atkin, and rich dark orange in Amrapali. The pulp of Chinwang at an immature state has an apple-like texture with less or no sour taste and can be eaten at the raw firm stage. The pulps of all the varieties are fibreless except in that of Tommy Atkin. Chinwang, Duncan and Tommy Atkin have prominent fruit neck while a slight fruit neck prominence was noticed in Irwin and Amrapali. Ventral shoulders of all the varieties are raised and rounded except in Duncan which ends in a long curve. Similar description for Tommy Atkin with oval to oblong fruit shape with a broadly round tip and dark red blush was made by Campbell (1973). Roundly oval fruit shape with yellow to golden yellow fruit colour, soft texture with fibreless flesh and steeply sloping shoulder was also reported by Sturrock (1969).

3.5. Flowering behaviour of the varieties

From the flowering habit (Table 5), Chinwang and Tommy Atkin come to harvest in the first week of June, Duncan and Irwin in the 2nd week of June while the check variety matures in the 3rd week of June. A similar observation was made for the three varieties Duncan, Tommy Atkin and Irwin by Crane, Balerdi, and Maguire (2006) where maturity was observed in the months of June and July. Amrapali has been observed to mature in the 3rd week of July (Chanana, Josan, & Arora, 2005).

Table 5. Observation of flowering behaviour of the varieties.

Variety	Initiation of flowering	50% flowering	Full Bloom	Fruit maturity
Chinwang	1st week of Jan	3rd week of Feb	Feb end	1st week of June
Duncan	2nd week of Jan	3rd week of Feb	Feb end	2nd week June
Irwin	Jan end	3rd week of Feb	1st week of March	2nd week June
Tommy Atkin	Jan end	Feb end	1st week of March	1st week of June
Amrapali	Jan end	3rd week of Feb	1st week of March	3rd week of June

4. Conclusion

All the four varieties are superior in quality as compared to the check variety (Amrapali) in most of the characters except in TSS. Results show no significant difference in the TSS. Fruit weight and pulp percentage are the important attributes that contribute to yield parameters and they are the recorded highest in Chinwang variety. Key consumer preference traits are fruit size and pulp percentage which all the four varieties showed excellent qualities with significant difference from the check variety. Consumers are also attracted to the size and colour of the fruits. The variety Irwin and Tommy Atkin have a red colour with medium size fruits. Further, all four varieties are high yielding as compared to check variety. It is apparent from this study that tree statures of all the varieties are short, and are suitable for high-density planting. Considering the results, all the four varieties can be considered as potential varieties that can be promoted in farmer's field. However, further evaluation through multi-location trials will help determine their performance under different climate and soil conditions. With good attributes in these varieties, study on their propagation methods can also be another area of research. Consumer preferences can also be studied as well as their market potential.

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Productivity and Preferences of New Potato Varieties and their Relationships in Five Districts of Bhutan

Pradeep Rai[b], Yadunath Bajgai[b], Tshering Lhadon[b], Lobzang[b], Sangay[b]

ABSTRACT

Potato (Solanum tuberosum L.) is one of the widely cultivated horticultural commodities in Bhutan. Over the years yields of potato at national level have stagnated due to degeneration of seeds and lack of appropriate varieties in the country. To address the yield stagnation issue, two potato varieties (Yusi Maap and Nasphel Kewa Kaap (NKK)) were introduced in recent years. To understand crop productivity and farmer's preferences of the new varieties this study was conducted in Bumthang, Chukha, Gasa, Haa, and Wangduephodrang districts from 2017 to 2018 using the field demonstration trials and participatory varietal selection method. Desiree, the popular variety was used as a control. On an average across years and dzongkhags, mean yields of the NKK and Yusi Maap were 10.4 and 10.5 tons/acre, respectively. Hence, yields of Yusi Maap and NKK were approximately 30% higher in comparison to Desire, which was 7.30 tons/acre on an average across the years and dzongkhags. Being one of the oldest varieties, Desiree consistently showed lower productivity compared to other two varieties due to seed degeneration and quality deterioration. Although the productivity difference between NKK and Yusi Maap is minimal (0.10 tons/acre) the preference votes of Yusi Maap was 1.3 times that of NKK indicating the higher level of preferences for Yusi Maap. There was positive significant relationship (P=0.02) between the potato productivity and the farmer's preference suggesting that higher productivity attracted more votes and lower productivity attracted lesser votes. Further higher productivity is also seen as a means to earn cash income for the household to achieve food security in terms of household's consumptions through the sale of potatoes. The findings provide a sound scientific basis to guide program implementers and policy-makers in terms of potato research and development at the national level.

Keywords: *Potato productivity; Farmer's preferences; Potato varieties*

1. Introduction

Potato (*Solanum tuberosum* L.) is considered as the fourth most important crop in terms of food consumption after wheat, rice and maize. According to Roder, Nidup, and Chettri (2008), the origin and cultivation of the crop dates back to as early as 8000 years ago in the high lands of South America. Later in the 16th century, Spanish explorers brought this magnificent plant to European continent merely as a botanical curiosity. However, within short span of time it spread

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throughout Europe providing cheap and abundant food alternative especially to workers of the industrial revolution. Eventually it gained popularity and became one of the most preferred food crops in Europe and gradually spread to other parts of Asia (Roder et al., 2008). Currently, potato is grown on an estimated 19 million hectares of farmland globally and its production stands at 378 million tons (Devaux et al., 2020). Although potato cultivation has been associated with developed countries historically, there is a shift towards developing nations in recent times with India and China surpassing and leading global productivity (Campos, 2020). Simultaneously, with progress in crop science potato crop is increasingly becoming more and more relevant in addressing the food and nutrition security and climate change impacts popularly in global south (Raymundo et al., 2018). Current breeding efforts through development of bio-fortified potato varieties have found to be supplementing various vitamin needs, mineral content, micro nutrition delivering high content of Fe, vitamin C and Zn and fight against hidden hunger and disease like anemia (Campos, 2020). Further, given its easy management and wider adaptation ability, potato crop has evolved as one of the most successful food crops for the entire world in terms of consumption (Devaux et al., 2020).

In Bhutan, potato was first introduced by Scottish explorer, George Bogle during one of his expeditions in the year 1774 (Roder et al., 2008). The members of the expedition planted the crop at their halting places while travelling from Buxa Duar through Chapcha to Thimphu. However, the authors also mentioned potato could have also reached Bhutan prior to his arrival because it was also found widely cultivated in northern India and in close proximity of Bhutanese southern border. It was only after late 1960s and early 1970s that Bhutan initiated formal potato development program through the assistance and support of the Swiss Agency for Development and Cooperation (SDC) (Roder et al., 2008). Although potato was newly introduced in the 60s, its cultivated areas exponentially increased by 10-20%, every year. The exceptional increase in adoption rate was accorded to farmer's own self-interest and introduction of relevant potato varieties. The important factors contributing to rapid adoption was attributed to market growth, improved road accessibility and access to improved potato varieties. Other factors relating to its rapid adoptability could be due to the crop's relatively longer shelf-life compared to other vegetables. Amongst other varieties introduced, the Dutch variety Desiree was found to be, by and large, widely accepted by the farmers due to its yield and superior agronomic performances during the early years of potato farming in the country (Roder et al., 2008, Roder, 2004). To improve potato industry, the Royal Government of Bhutan then initiated several intervention measures, primary amongst them being the introduction of potato auction yard through the Food Corporation of Bhutan (FCB) in 1980 (Roder, Nidup, & Wangdi, 2007). Since then, the concept of downhill movement of potato seed quality began in the country. According to RSD (2020) the present harvested area under potato cultivation is estimated at 10,342 acres out of total cultivated area of 189,499. 37 acres in the country.

The crop which was once commonly grown as a homestead vegetable is now one of the most widely cultivated, traded and consumed crops in Bhutan (Bajgai et al., 2018). Due to its physiological considerations, cultivation of potato as a commercial crop is mainly concentrated at elevations between 1500-3000 masl. Nonetheless, given its wider adaptation capacity and easy management, it is also found cultivated in areas as low as 200 masl - mostly covering the southern dzongkhags of Bhutan - as winter crop for self-consumption (Roder et al., 2008). Potato is not considered as a staple crop yet in Bhutan, however, 21% of the total farming households depend on potato farming for their daily livelihood comprising 34,000 households of the total 1, 63,001 households in Bhutan (RSD, 2017; NSB, 2017). The income received from the sale of potato by farmers directly help purchase household items, food essentials and render livelihood support. Further, Bajgai et al. (2018) reported that the revenue generated through export of potato amounting to Nu 797 million in 2016 was one of the highest among horticulture commodities in the country. Similarly, RSD (2020) revealed that potato alone generated a revenue of Nu 709.81 million through the export of 30,277.08 MT in 2019, surpassing the combined revenue earned through the sale of vegetables and pulses at Nu 169.15 million only.

Although potato farming significantly contributes to national revenue and livelihood support, there is a declining trend in potato productivity as observed over the decades mainly due to lack in diversity of desired potato seed varieties and degeneration in seeds of the existing varieties (Bajgai et al., 2018). According to FAO (2014) the productivity of potato in Bhutan stood at 8.9 t/ha whereas the neighbouring countries like Bangladesh, India and Nepal reported yields of 19.3, 22.9 and 13.7 t/ha, respectively which is comparatively higher than that of Bhutan's. Owing to this and given the opportunities for expansion of cultivation area and increase productivity, the National Potato Program with the Department of Agriculture released potato clone accession CIP 393077.159 as Nasphel Kewa Kaap variety (NKK) in 2014 and accession number CIP 392797.22 as Yusi Maap in 2017. These clones were imported from the International Potato Centre (CIP) to generate higher yielding and better varieties as an intervention to address the stagnation issue of potato productivity. Following their release, the new varieties were demonstrated through field trials in major potato growing dzongkhags of Bhutan with the intention of transferring the new varietal technology to the farmers.

However, within a short span of time since the release of these varieties, Yusi Maap gained huge popularity and there is an overwhelming demand from growers in the country. Against this backdrop, this paper attempts to understand as to why farmers are interested in the newly released Yusi Maap potato variety as compared to other varieties and, also determine farmers' preferences of the released variety. The missing information sought would help design appropriate planning and interventions for researchers and relevant stakeholders in the future to enhance potato industry in the country. The objectives of this paper are:

1. Compare the productivity of the newly released potato varieties of Yusi Maap (YM) and Nasphel Kewa Kaap (NKK) with the existing commonly grown and popular variety Desiree in Bumthang, Chukha, Gasa, Haa and Wangdue over three years period from 2017 to 2019.

2. To assess the farmer's preference of the three varieties through participatory selection procedure in the aforementioned five dzongkhags from 2017 to 2019.
3. To determine the relationship between the productivity and the farmers preference votes.

2. Materials and Method

2.1. Description of the Research study Area

The National Potato Program (NPP), National Centre for Organic Agriculture (NCOA) – Yusipang, in collaboration with the dzongkhags/geogs disseminated newly released potato varieties to major potato growing parts of the country as part of its continuous evaluation and production assessment program. In 2017 potato field demonstration trials were set up in the main potato growing dzongkhags of Trashigang, Mongar, Pemagathsel, Bumthang, Paro, Chukha, Thimphu, Gasa, Haa and Wangdue. The idea was to disseminate growing technology to growers involving them throughout the growing season. However, yield and farmer's preferences were assessed consistently over three years from 2017 to 2019 in Bumthang, Chukha, Gasa, Haa, and Wangduephodrang dzonkghags only. Therefore, only these five dzongkhags were considered for the paper. The demonstration sites selected in consultation with geog agriculture extension staff and local government officials. The site was in a single household in a village and in the following year the demonstration was selected in another village according to suitability of the crop. Each demonstration site was provided with 50 kg seeds of the new two varieties (Yusi Maap and NKK). Desiree was provided only if the locally grown seeds were degenerated. Suphala fertilizer, irrigation pipes and other equipment were provided during the demonstration.

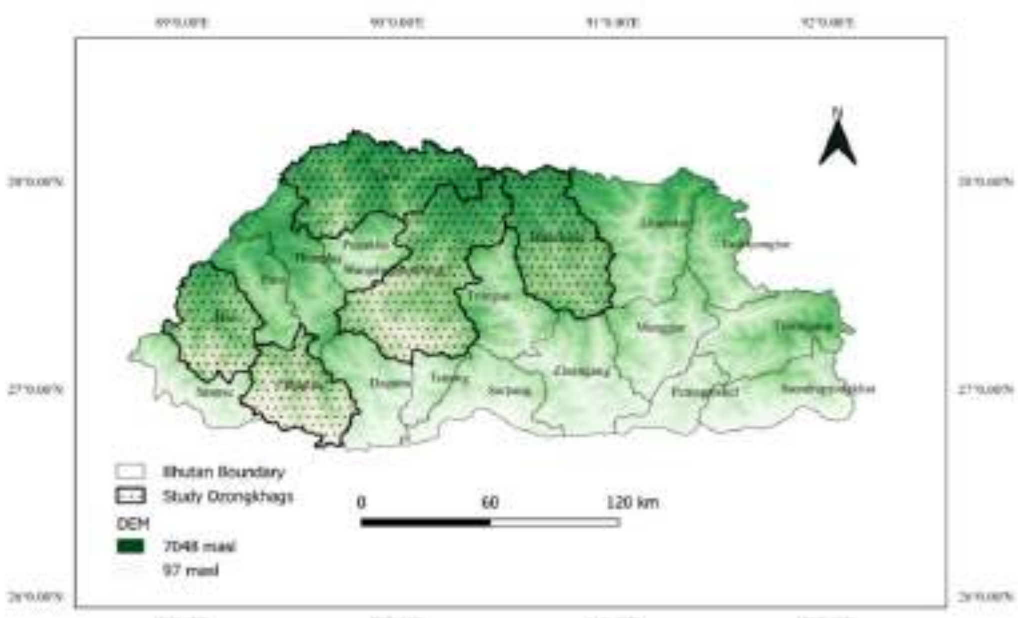

Figure 1. Location of potato demonstration dzongkhags.

2.2. Description of the major Agro-ecological zone for potato farming in Bhutan

There are six major agro-ecological zones identified in Bhutan (described in Table1). Depending on these agro-ecological zones, there are three major dominant land use categories and cropping patterns identified in Bhutan (NSSC, 2010). According to Katwal and Bazile (2020), the major cropping systems under different agro-ecological zones are:

1) Potato, wheat and apple-based farming in Kamzhing (dry land) under cool and warm temperate zone wherein other crops such as vegetables, mustard and buckwheat are rotated or intercropped in orchards.
2) Maize based cropping system predominates with cereals such as millets, buckwheat followed by vegetables, legumes and oilseeds in dry and humid subtropical areas and in warm temperate zones *Kamzing* (dry land).
3) In the terraced wetland (Chhuzhing) under warm temperate zone, farmers mostly grow a single crop of high-altitude irrigated rice with some farmers rotating peas, potato, oat and wheat as fodder after rice.

Based on these prevailing agro-ecological zones, majority of the farmers practice self-sustaining, integrated agricultural production system where they grow a variety of crops under different farming practices and rear livestock to meet their food security (Katwal et al., 2015). Owing to the topography dominated by high mountains and deep valleys there is huge variation in the micro-climate which calls for location specific crops and varieties (Katwal et al., 2015). Accordingly, within these given agro-ecological zones, potato farming is specifically spread and mainly concentrated in the warm temperate to temperate region as a semi-commercial farming undertaking. The concentration in these regions can be attributed due to the favorable conditions available during the crop's morphological and physiological development (Roder et al., 2008).

Table 1. Major agro-ecological zones of Bhutan.

Major Agro-ecological Zones	Altitude (masl)	Temperature (degree Celsius)		Mean Rainfall (mm)	Geographical area (%)
		Max	Min		
Alpine	3600.1 - 7500	12.0	-1.0	<670	28.56
Cool Temperate	2600.1 - 3600	20.0	1.0	650-850	23.89
Warm temperate	1800-2600	26.0	1.0	650-870	18.61
Dry Subtropical	1200.1- 1800	29.0	3.0	870-1200	13.11
Humid Sub tropical	600.1-1200	33.0	5.0	1200-1500	10.23
Wet Subtropical	94. - 600	35.0	12.0	2500-5500	5.60

Adapted from RSD (2017)

Table 2. Land use categories and major cropping pattern.

Major Agro-ecological Zones	Altitude (masl)	Land use types		Major crops (Cropping pattern)
		Kamzhing (dry land)	**Wetland (Chhuzing)**	Horticulture/Plantation
Alpine	3600.1 - 7296	Pasture	Absent	Absent
Cool Temperate	2600.1 - 3600	Barley-fallow, Potato-Turnip	Absent	Apple
Warm temperate	1800-2600	Potato-Buckwheat, Potato-Turnip, Wheat/Barley-Buckwheat, Potato-Wheat/Barley, Vegetables- Wheat	Rice- Fallow, Rice-Potato, Rice- Peas, Rice- Wheat	Apple, Walnut, Pear, Peach, Plum
Dry Subtropical	1200.1-1800	Maize and Potato, Maize and Soybeans, Maize and Mustard, Maize and Barley, Maize and fodder Oat, Maize and Buckwheat, Chilli- fallow, Vegetables and Wheat	Rice- wheat, Rice-Mustard, Rice- Chilli, Rice- Vegetables	Apples, Pears, Peach, Kiwi, Large Cardamom
Humid Sub tropical	600.1-1200	Maize- Maize, Maize-beans (Rajma), Maize-Millet	Rice-Fallow, Rice-Mustard	Citrus, Large Cardamom
Wet Subtropical	94. - 600	Maize- Mustard, Maize- Maize, Maize-Grain Legumes (Black Gram, rice bean, broad beans) and Maize and Millet	Rice- Fallow, Rice-Maize, Rice-Wheat, Rice-Sesbania	Arecanut, Mango, Avocado, Banana, Litchi

Adapted from Katwal and Bazile (2019)

Table 3. Description of three potato varieties under demonstration trials in five Dzongkhags.

Name of Potato Variety	Accession no and year of release	Characteristics and Agro-ecology
Desiree	CIP800048 & 1988	-Red skinned, relatively high yield potential, -Agro-ecological/elevation: 1000-2000 meter above sea level (masl), -130-140 days to maturity
NasphelKewaKaap (NKK)	CIP393077.159 & 2014	-High yielding, pink eye and resistant to late blight - All agro-ecologies -160-180 days to maturity
Yusi Maap (YM)	CIP392797.22 & 2017	-High yielding, red skinned, moderately resistant to late blight, high content of micro nutrient of Ca, Zn and Fe; Mid and high altitudes; 130-140 days to maturity

Table 4. Physical appearance of different potato varieties.

Name of Varieties	Plant Physical Appearance	Tuber appearance
Desiree		

Nasphel Kewa Kaap (NKK)

Yusi Maap (YM)

2.3. Data Collection and Analysis

Purposive sampling technique was employed for identification of households where field demonstration sites were located. The households were identified in consultation with local government officials at the geog level including the Dzongkhag Agriculture Officers (DAOs) and Geog Agriculture Extension Officials (GAEO). Both primary and secondary data were used in this paper. Primary data on yield of potato varieties were collected from those households that were involved in cultivating and demonstrating the newly released potato varieties from 2017 to 2019. Primary data of preference votes on potato varieties were generated from the demonstrations and training programs organized at the sites in all five dzongkhags. Participatory Variety Selection (PVS) was employed at harvesting stages in all the sites. While collecting the information, gender-responsive voting methodology developed by the International Centre for Potato (CIP) was also employed. In the process of voting, both men and women were given six numbers of corn seeds and bean seeds, respectively. Each farmer (male and female) participant cast three votes for the most desired varieties/clones. Similarly, votes were cast for the second best with two votes and the third with only one vote depending on the physical appearance of the different varieties displayed (Haan et al., 2019). Votes were cast based on farmer's preference on the basis of yield, tuber sizes and distribution, tuber color, perceived marketability, resistance to late blight and nutrient content of the potato variety. The varieties receiving maximum numbers of total votes determined the best variety.

Secondary data on trends in potato production and export value from 2012 to 2017 in Bhutan were obtained from the www.moaf.gov.bt (DoA, 2012, 2013, 2014, 2015, 2016, RSD, 2017) and www.mof.gov.bt (DRC, 2014, 2015, 2016, 2017, 2018). The information collected both from primary and secondary data were organized systematically. In order to analyze the relationships between the productivity of different varieties of potato with that of votes cast by the farmers, Pearson's correlation was employed while variation in the productivity was analyzed and derived through the use of standard deviation and standard error of each variety from each dzongkhag. Similarly, comparative yield assessment of three potato varieties, viz. Yusi Maap, Nasphel Kewa Kaap (NKK) and Desiree were carried out and produced in the form of graph through R software Version 3.6.6 as well as Microsoft Excel version 2010. A total of 947 farmer participants were involved in the study that spanned from 2017 to 2019 comprising of five main potato growing dzongkhags (Figure 1).

3. Results and Discussion

Figure 2 presents the overall total potato production scenario in the country from 2012 to 2017. The total production remained fairly stable with the lowest production recorded at 43,000 tons and highest at 57,223 tons over these years. However, the export value earned and the quantity produced did not directly correspond due to the prevailing price differences determined by market forces that in turn was subject to variation in the production in the neighboring states of India. In 2014, the price of Bhutanese potato at the FCBL auction yards was particularly high due to the slightly lower production in neighboring Indian states which thereby increased the demand for Bhutanese potatoes. In some years prices dropped, indicating the decrease in demand as a result of the high productivity in neighboring Indian states. This indicates the dependency on the vagaries of external markets where prices are beyond the country's influence and control, thus adversely affecting potato growers and their livelihood.

There is the need to explore assured market for long term sustainability of potato farmers. Earlier study carried out by Roder et al.(2007) also emphasized the urgent need to develop clear road map to benefit potato farmers as well as the nation. Although majority of the farmers practice subsistence farming, potato has singularly transformed Bhutanese agriculture from subsistence to emerging market-oriented farming, supporting livelihoods of a large number of farmers, especially living at elevations between 1500-3000 masl (Roder, 2004). The analysis reveals that production has been fairly stable over the span of six years indicating the slightly stagnated nature of potato productivity.

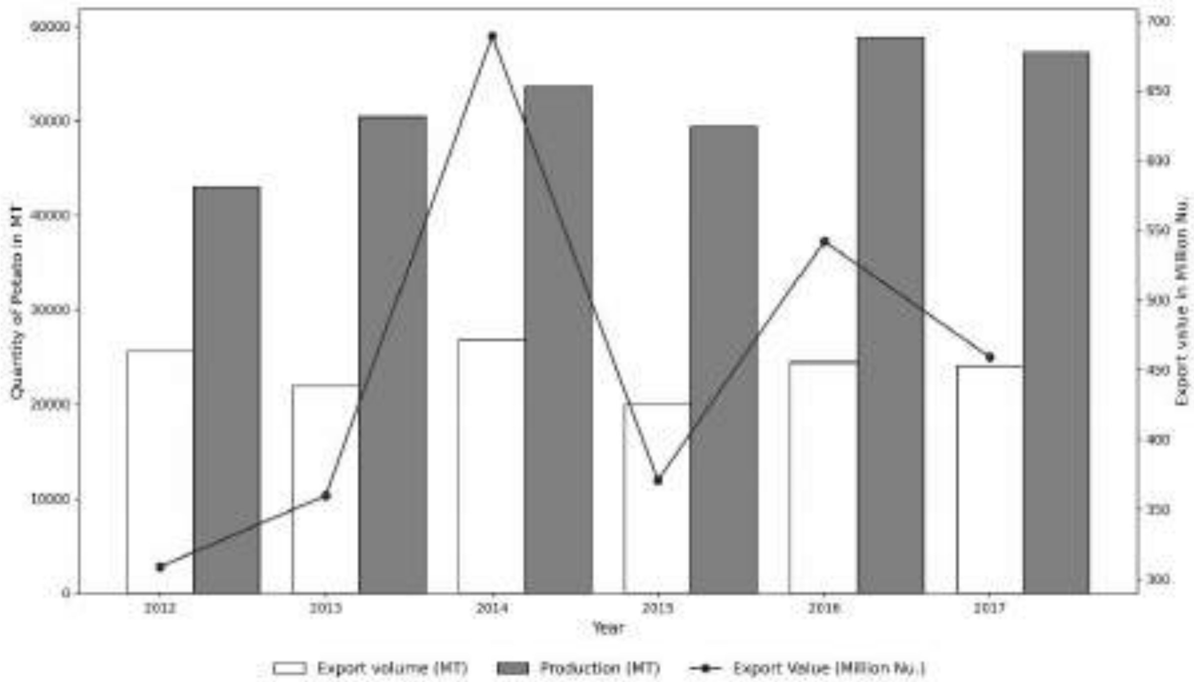

Figure 2. Trend in potato production and export from 2012 to 2017 in Bhutan.

The comparative mean productivity of three different potato varieties (Desiree, Nasphel Kewa Kaap and Desiree) in five potato growing dzongkhags (Bumthang, Chukha, Gasa, Haa and Wangdue) over the three years from 2017 to 2019 is presented in Figure 3. On an average, across the dzongkhags mean yields of NKK and Yusi Maap were 10.42 and 10.52 tons/acre, respectively. The mean yield difference was as minimal as 0.10 tons/acre between these two new potato varieties whereas Yusi Maap showed slightly higher average yield than NKK**.** However, Desiree variety yielded 7.30 tons/acre on an average across dzongkhags and over the years. Being one of the oldest varieties, Desiree consistently showed lower productivity compared to the other two varieties (Figure 3). Even though Desiree was a popular variety in the past, overall crop performance of YM and NKK in terms of yield was about 30% higher. Standard deviation values for Desiree showed greater variation compared to other two varieties particularly in 2017 indicating lower yield stability. As stated by Thomas-Sharma et al. (2015), the lower productivity in potato varieties could be due to the degeneration of seed quality and inadequacies in timely seed replacement, further aggravated by accumulation of pathogens and pests in the planting materials. They further observed that small scale farmers mostly from developing world continues to plant seed tubers acquired through informal seed systems that is either produced on-farm or acquired from neighbors or local markets. Similarly, Bajgai et al. (2018) also reported that the yield of Desiree variety and other old varieties have consistently shown stagnation in productivity over the years due to limited availability of superior potato seed quality and as a result of degeneration in the quality of seed potato.

However, this is also an indication of the opportunities for the National Potato Program to augment and explore potato production through diversifications of desired potato seeds. The consistency in better performance in productivity by the two new varieties is due to their fairly new genetic seed composition and vigor which is comparable with the current productivity reported in neighboring countries like Bangladesh, India and Nepal (Bajgai et al., 2018). According to the author, although, NKK showed similar comparative productivity trend to that of Yusi Maap in the past studies, the acceptability of this variety by the farmers was however, lower when compared with Yusi Maap. This is attributed to the NKK being a white-skinned variety fetching market prices lower than Yusi Maap. Generally, growers prefer red-skinned variety like Yusi Maap because consumers prefer them and are higher in demand (Roder et al., 2007). Moreover, red-skinned varieties are offered higher prices by the buyers at the FCBL auction yards in Phuentsholing, Gelephu and Samdrupjongkhar. A study carried out in Nepal also found that farmers were less receptive to white varieties even if they were high yielding (Upadhyay, Ghimire, Acharya, & Sharma, 2020).

Unlike Yusi Maap, NKK possesses higher resistance to late blight disease and thereby has the potential to address food and nutrition security and improve livelihood of farmers in the country (Bajgai et al., 2018). Similarly, the analysis of mean productivity of three varieties across all the five research study sites at dzongkhag level (Figure 4) also show the similar performance pattern with Desiree's performing consistently lower than the two varieties. The overall mean productivity difference of Desiree from that of NKK was 3.7 tons/acre, and 3.12 tons/acre lower than that of Yusi Maap. And analysis between the newly released varieties, NKK and Yusi Maap found the average yield difference as only 0.052 tons/acre when compared across five dzongkhags from 2017 to 2019. Based on the current yield trend and market price if we could replace all existing Desiree variety with Yusi Maap, our national productivity will increase by 30.6% with corresponding increase in revenue generated through export as well. The scenario is similar for NKK variety. Further, due to its high adaptive capacities and easy management, there is huge opportunity to increase production through staggered cultivation practice. Staggered cultivation in the three agro-ecologies of low-altitudes, mid-altitudes and high-altitudes has the potential to supply potato to the country for most part of the year.

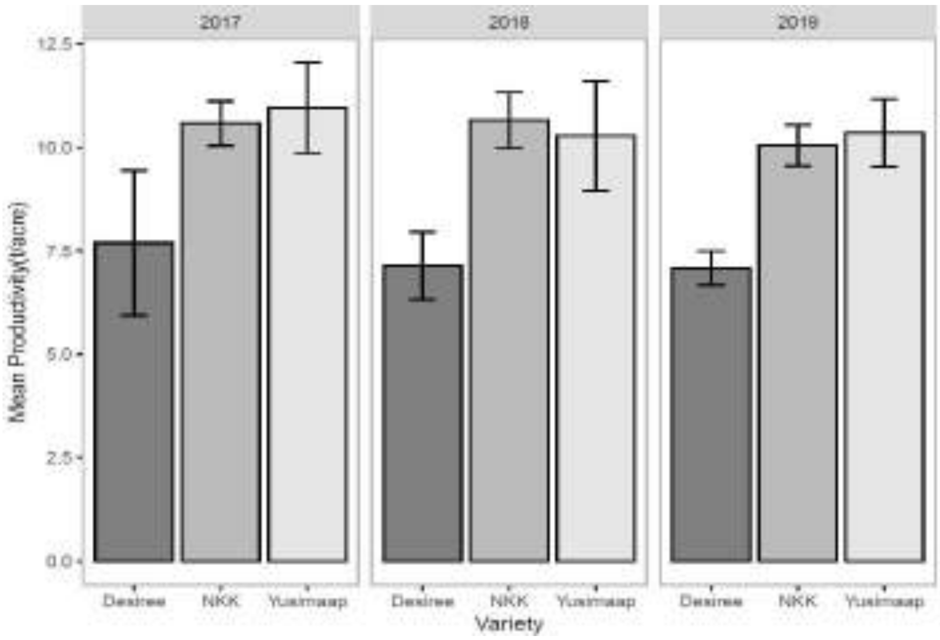

Figure 3. Mean yield of three potato varieties from 2017 to 2019 across five dzongkhags. Error bars show SE of means calculated for each year.

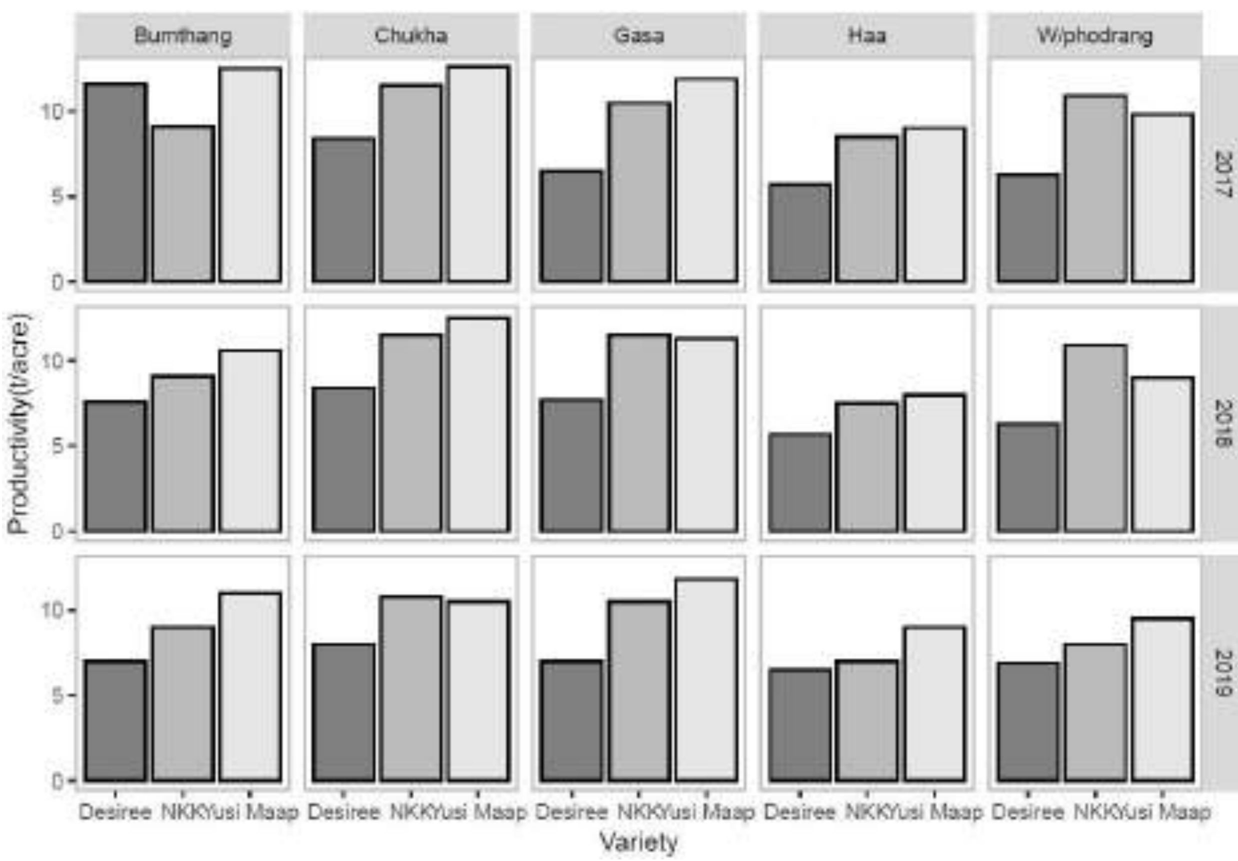

Figure 4. Dzongkhag-wise yield of three potato varieties from 2017 to 2019.

Similarly, Figure 5 explains the preference votes cast by farmers for the three potato varieties in the five research study sites from 2017 to 2019 following participatory varietal selection methodology (Haan et al., 2019). The data represents votes cast by both male and female farmer participants who took part in the field day-cum-training program organized at the demonstration sites. Both the new potato varieties received higher number of votes in comparison to the old variety Desiree in all the three years. In 2017, Desiree received only 473 total votes whereas NKK received 676 and Yusi Maap received the highest total votes of 849. Similarly, in 2018 the total votes for Desiree was 494, NKK 703 and 927 for YM. In the following year (2019), Desiree received 400, NKK 492 and YM 668 votes. Across the sites and years, Desiree received 79 and 37% less votes in comparison to Yusi Maap and NKK, respectively. In stark contrast, although the productivity difference between NKK and Yusi Maap is minimal (Figure 3), the preference votes for Yusi Maap was 1.3 times that of NKK, clearly indicating Yusi Maap is preferred the most. This reflects farmer's preference for red-skinned variety due to better marketability as red-skinned varieties are preferred by consumers as well as by the bidders at all the FCBL auction yards (Roder et al., 2007). Likewise, a study conducted in Nepal also reported thatfarmers were more receptive to new red potato varieties than white-skinned ones, and that adoption rate was found to be low for white-skinned than red skinned varieties (Upadhyay et al., 2020). With the similar agro-ecological environment between Bhutan and Nepal, our farmers seem to have a similar tendency with regards to adoption of new technologies. Additionally, a study carried out by (Bajgai et al., 2018), also found report that red variety Yusi Maap was found to be more receptive by the farmers as compared to NKK although it has similar productivity potential.

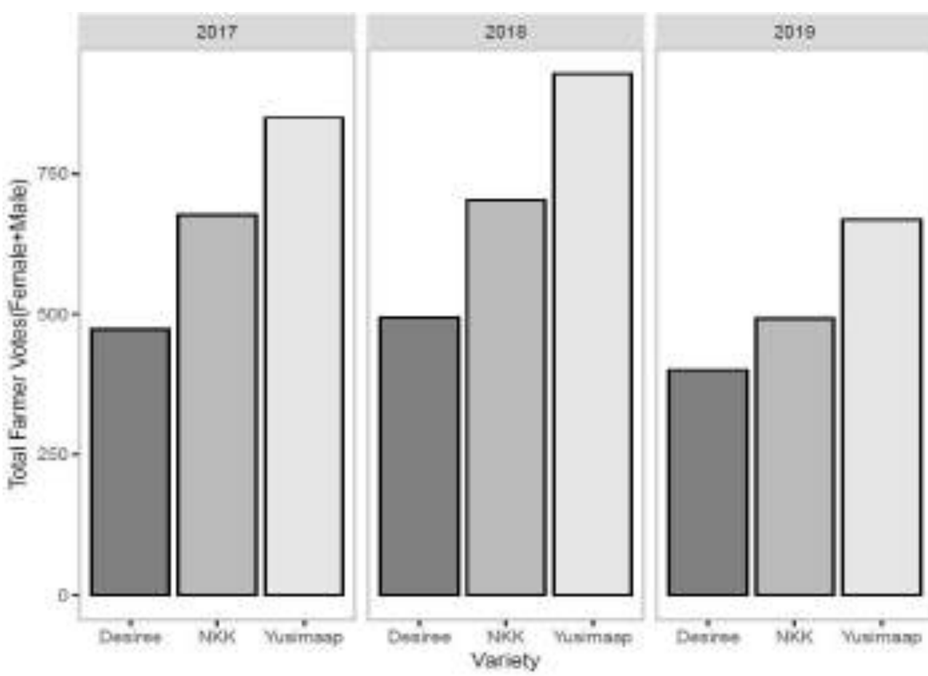

Figure 5. Preference votes casted by farmers (Female and Male) during the participatory varietal selection (PVS) process over the choice of potato varieties.

Statistical relationship between the yields and preference votes was tested. To determine the relationship Pearson's correlation analysis was performed. The correlation model between potato productivity and the farmer's preference votes showed significant association (R= 0.35, P=0.02) indicating potato productivity is positively related to the number votes cast for the three potato varieties (Figure 6). In other words, the higher the potato productivity, the higher is the preference votes cast, meaning higher productivity attracted more votes and lower productivity attracted lesser votes.

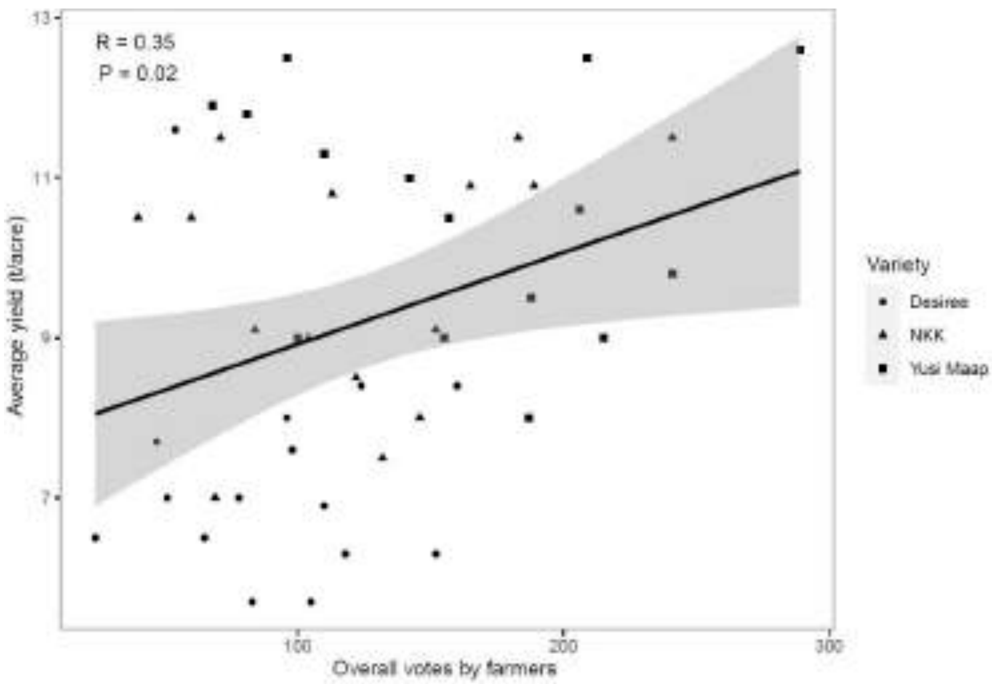

Figure 6. Relationship between the farmer's preference votes and yields of three potato varieties in five districts from 2017 to 2019.

Bajgai et al. (2018) in their study on farmer's selection criterion that included 1) high yielding, 2) oblong shape, 3) floury texture, 4) medium and uniform size, 5) high nutrients content 6) scab resistant 7) chipping quality, ranked from 1 being highly preferred to 7 being the least preferred found the highest number of votes were cast for the highest yielding varieties/clones and the least for the chipping varieties. Further, the study revealed that even if the varieties possess other important attritubes like chipping qualities, farmers would still first prefer high yielding varieties amongst other in general. This could be due to the concerns of feeding their family first which principally reflects the importance of the food availabilty both in quantity and quality amongst farmers in the global south unlike their northern counterparts who normally opt for easy, fast and nutrtitive food (Devaux et al., 2020). Moreso, the higher productivity in potato is also seen as the direct and most certain means to earning cash income, and an importance parameter in assuring househild food security.

4. Conclusion

In summary, the study showed that, mean yield of Desiree is consistently lower than that of the NKK and Yusi Maap. Yields of Yusi Maap and NKK were approximately 30% higher in comparison to Desiree. Being one of the oldest varieties, Desiree consistently showed lower productivity compared to other two varieties due to seed degeneration and quality deterioration. Across the sites and years, Desiree received 79% and 37% less votes in comparison to Yusi Maap and NKK, respectivly. Although the productivity difference between NKK and Yusi Maap was minimal (0.10 tons/acre), the preference votes for Yusi Maap was 1.3 times that of NKK indicating the higher level of preferences for Yusi Maap because of its marketable appearance. Although farmers' preference could also be related to colour, marketability, resistance to late blight, tuber sizes , empirically this study found a positive significant relationship between the potato productivity and the farmer's preference votes suggesting that the higher productivity attracted more votes and lower productivity attracted lesser votes. The findings of this study is likely to provide a sound scientific basis to guide program implementers and policy-makers in terms of potato recearch and development in Bhutan.

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Effect of Wood-derived Biochar as Growth and Development Medium for Kiwifruit Seedlings

Birkha Bahadur Tamang[c], Thinley Dorji[c] and Lhap Dorji[c]

ABSTRACT

Application of biochar as a soil amendment has gained attention as a sustainable technology for environmental remediation. However, information on its use as a growing medium amendment in Bhutanese context is presently unavailable. Hence, this study assessed biochar's physical and chemical properties as well as the response of kiwifruit seedlings to biochar-amended media. Kiwifruit seedlings were grown in different locally available organic growing media containing different quantities of wood biochar. Seven different media namely: treatment 1 (compost, 100%), treatment 2 (wood biochar + compost, 25%: 75%), treatment 3 (wood biochar + compost, 50%: 50%), treatment 4 (wood biochar + compost, 75%: 25%), treatment 5 (wood biochar, 100%), treatment 6 (wood biochar + rice husk, 50%: 50%); and treatment 7 (wood biochar + vermicompost, 50%: 50%) were used. Treatments containing biochar recorded higher pH, implying biochar addition in acidic soil reduces liming need. Kiwifruit seedlings responded significantly to the biochar-amended medium as compared with the unamended controls. This was reflected in the plant growth parameters: plant girth, plant height, taproot length and the number of lateral roots. A significant effect ($P \leq 0.05$) in seedling girth (6.60 mm), height (68.60 cm), taproot length (44.40 cm), and lateral roots (25 numbers) was observed in treatment 2 (amended with 25% wood biochar). Likewise, medium prepared with 100% wood biochar (treatment 5) resulted in a minimum effect on all parameters. Our study reveals that biochar amendment can be a method for generating carbon offsets, but at quantities exceeding 25% of the total medium, no significant effect on the overall growth of plants is achieved.

Keywords: *Kiwifruit seedling; Medium; Wood biochar; Organic; Plant parameters*

1. Introduction

Intensive agricultural practices in response to global food requirement have led to the use of various agrochemicals. In reckless pursuit of enhancing crop productivity, a tremendous amount of antecedent soil organic carbon is being lost into the atmosphere (Stavi & Lal, 2012). As a result, the negative consequences of climate change like global warming, melting of ice-caps, submerging of land under water bodies, unpredictable weather pattern, incessant outbreak of pests and diseases have been intensifying. These ill effects on the environment have forced mankind to look for

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environment-friendly organic nutrient supplement alternatives. Biochar application provides opportunity as one of the organic nutrient supplement choices in farming.

Biochar is a solid carbonaceous residue made by burning biomass under oxygen-free to oxygen-deficient conditions. Wood chips, crop residues, nut shells, seed mill screenings, algae, animal manure and sewage sludge are some of the many feedstocks used in biochar production. Many experts describe the use of biochar as an age-old solution to modern problems (Mohan et al., 2018; Schouten, 2010).

Physicochemical and biological properties of the biochar depend on the type of materials used and the temperature at which pyrolysis is undertaken. In a study by Gul et al. (2015), biochar produced from crop residues at lower temperatures (< 600 ^{o}C) had higher pH and cation exchange capacity with more microorganism content than biochar produced from wood-derived feedstock pyrolyzed at higher temperatures (> 600 ^{o}C). Biochar produced at higher temperature have a larger surface area and higher porosity as reported by Mohanty et al. (2013).

According to Mohan et al. (2018), the Amazon basin contains huge amounts of sequestered carbon. In light of climate change mitigation by sequestrating recalcitrant carbon in the soil, biochar usage in farming could be a novel technology. Biochar could be potentially low cost, easily available, and environment-friendly constituent of growing media although it may not contain as many nutrients (Nemati, Simard, Fortin, & Beaudoin, 2015). Biochar helps retain nutrient and moisture of the growing medium. They observed only 11% nutrient leaching in growing media treated with biochar.

In Bhutan, the use of biochar as a soil amendment and growing media is gaining popularity. Likewise, organic agriculture has been given high priority by the Royal Government of Bhutan in the 12th Five Year Plan. Biochar has been released as a technology at the 2nd sitting of the Technology Release Committee (TRC) of the Department of Agriculture, Ministry of Agriculture and Forests convened on 29 May 2020 (DoA, 2020). Although charcoal and ash from our household hearths have been applied into the soil for ages in the country, proper assessment of their effects on soil and crop yield has not yet been studied.

Therefore, this study was conducted to assess the physico-chemical properties of media amended with different levels of biochar and their effect on the growth of kiwifruit seedling.

2. Materials and Method

2.1. Study area

This study was carried out on the research farm of the Agriculture Research and Development Center (ARDC-Wengkhar), Mongar, from June 2019 to May 2020. The site is located at 1732 m above sea level between 27^{o} 16' 09.6'' N and 91^{o} 16' 19.9''E. It falls under dry sub-tropical zone and faces north-west. The area receives close to 1000 millimetres of annual rainfall.

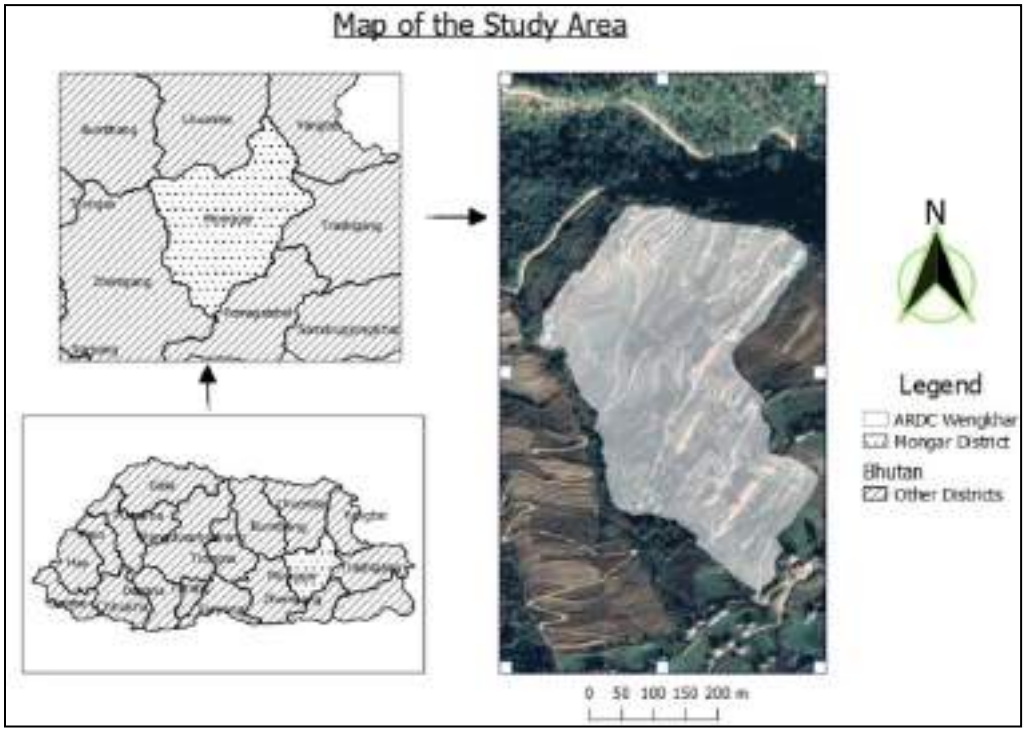

Figure 1. Map of the study site.

2.2. Experiment Setup

The experiment was laid out in random complete block design (RCBD) with seven treatments replicated three times each. About two months old or 3-leaf stage kiwifruit seedlings were transplanted in 40 * 40 * 80 mm poly tubes. Each treatment had 15 plants of which five seedlings were tagged for routine data collection.

Wood biochar was produced from pruned branches and twigs of assorted fruit plants like citrus, pear, peach, plum, chestnut and kiwifruit through the pyrolysis process. The feedstock was made into small pieces. The method adopted was a traditional which involved letting wood chips to burn underground overlayered with a cover of a thick layer of green leaves and soil. It was then ground into fine powder manually using a piece of wood or stone, followed by sieving using a wire mesh with an eye size of 0.1 * 0.1 mm. The growing media mix preparation was done adding wood biochar to different proportions in separate locally available organic substrates as follows:

i) Compost (100%)

ii) Biochar + compost (25%: 75%)

iii) Biochar + compost (50%: 50%)

iv) Biochar + compost (75%: 25%)

v) Biochar (100%)

vi) Biochar + rice husk (50%: 50%)

vii) Biochar + vermicompost (50%: 50%)

The polytubes were filled with media mixtures, and 2 to 3-leaf stage seedlings were transplanted after dipping their roots in copper oxychloride solution for an hour. Before planting the seedlings, records on plant height, stem girth, taproot length and the number of lateral roots was maintained.

Seedlings which could not survive were replaced every forth-nightly for three months and data recorded. The temperature of the media mixes was recorded with a soil thermometer. Manual irrigation was provided using watering can every two days up to three months, followed then by application of water depending on seedling and weather condition.

Plant height and stem girth data were collected after every three months. At the end of one year, data on taproot length and number of laterals were recorded by destructive sampling method.

2.3. Data collection and analysis

During the study period of one year, data on seedling height and girth were recorded quarterly. Physiochemical properties of the different potting mixes were assessed at the Soil and Plant Analytical Laboratory of the National Soil Services Center (NSSC), Department of Agriculture, Simtokha, Thimphu. Data analyses were conducted using MS Excel spreadsheet and Statistical Tool for Agricultural Research (STAR) software. After checking for normal distribution of the data, one-way analysis of variance (ANOVA) was conducted. The significance level of the treatments was tested at P level 0.05.

3. Results and Discussion

3.1. Laboratory analysis

Media mixes of different proportion had different chemical properties affecting plant nutrient availability ultimately. Treatments amended with biochar resulted in higher pH. The higher pH in media mix treated with biochar was due to increased temperature during pyrolysis that resulted in the removal of the carboxyl and hydroxyl groups of compounds from the charcoal. The media prepared with biochar alone had the highest of pH (10.17) while compost had neutral pH. Likewise, available potassium was the maximum in the treatment prepared with 50% each of biochar and vermicompost followed by the treatment composed of biochar and rice husk (50%: 50%). The compost media had the highest nitrogen content while treatment with 100% biochar showed the lowest N. Table 1. below presents the chemical properties of the different media mixes.

Table 1. Chemical characteristics of the treatments.

Treatments	Composition	pH		Available K	N
		H2O	KCL	*mg/kg* (Bray)	Percentage (%)
1	Compost (100%)	7.01 M	6.95 M	331.36 vH	0.48 M
2	Biochar + compost (25%: 75%)	7.43 vH	7.35 vH	309.47 vH	0.25 M

3	Biochar + compost (50%: 50%)	8.45 vH	8.25 vH	242.99 vH	0.19 L
4	Biochar + compost (75%: 25%)	9.58 vH	9.02 vH	171.58 vH	0.18 L
5	Biochar (100%)	10.17 vH	9.58 vH	284.11 vH	0.17 L
6	Biochar + rice husk (50%: 50%)	9.35 vH	8.47 vH	333.03 vH	0.18 L
7	Biochar + vermicompost (50%: 50%)	7.44 vH	7.00 M	338.40 vH	0.32 M

Ratings: vL-Very low, L - Low, M-Medium, H-High, vH-Very high

3.2. Seedling mortality and re-transplantation

Seedling mortality was recorded weekly and replanted until one month of the trial establishment. In the 1st week, seedling survival for all the treatments was almost the same. However, in the 2nd week, we re-planted 32 and 34 numbers of seedlings in treatment 2 and 3, respectively. The percentage of seedlings required to re-transplant gradually dropped from 75.16% in the 3rd week to 55.07 % in the fourth week where a minimum of 2 numbers of seedlings was replanted in treatments 4, 6 and 7. Maximum of 9 numbers of seedlings were planted in treatment 5. It was observed that the seedling mortality trend was the maximum in the media mixes treated with biochar. This could be attributed to higher pH values which the addition of biochar to the mixes resulted in, which subsequently led to poor nutrient availability. Also, high seedling mortality was due to excessive amount of water retained by biochar causing root suffocation and decay.

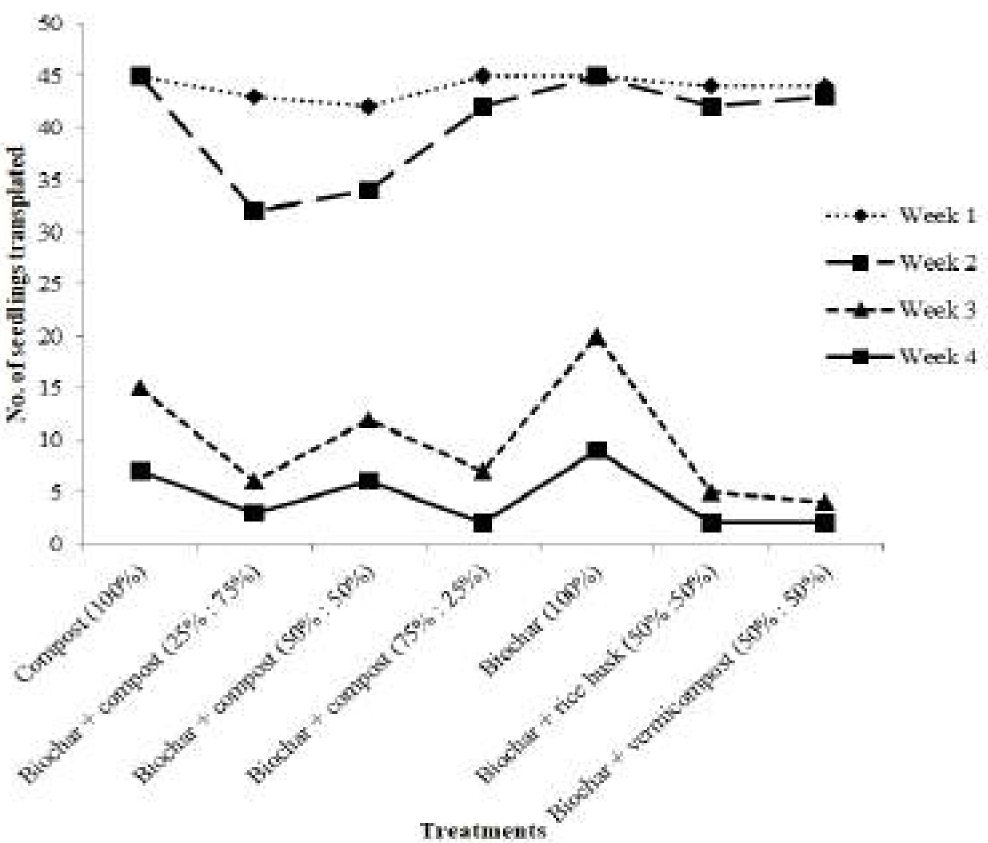

Figure 2. Number of seedlings replanted in different treatments in one month.

3.3. Above-ground seedling growth

Plant girth at 10 to 15cm from the root collar is the determining factor for a rootstock to be considered as having reached the optimum grafting stage. The rootstock is considered to have attained graftable size when its girth attains pencil size (more than 6.5 mm in diameter). It was measured using a digital vernier calliper (model: CD-S20CT) every after three months. The effect of biochar amendment on both girth and height of the seedlings was similar. Media mixes with biochar had a significant effect ($P \leq 0.5$) on the seedling girth. The maximum girth of 6.69 mm followed by 6.40mm was recorded in biochar and biochar 50% + vermicompost 50%, respectively. Likewise, the minimum effect was seen in treatment 5 (2.06 mm) where the medium was 100% wood biochar.

In a study by Ismail and Iberahim (2003), the addition of 5% biochar by weight in potted tomato had a significant effect on plant physiology and yield due to improved moisture content. Similarly, Khan et al. (2015) had observed increased production of turnip with the addition of 2% biochar than 5% when added into soil. Likewise, Graber et al. (2010) also found a significant effect on the yield of tomato and pepper with an addition of 1 to 5% by weight of wood-derived biochar into coconut fibre. Aller et al. (2018) also noted that the lower rate of biochar application yielded better corn yield than higher rates. Other factors responsible for poor growth in seedlings with the increase in biochar proportion could be due to a decrease in nitrogen uptake by the seedlings as reported by Rondon, Lehmann, Ramírez, and Hurtado (2007). Their study reported that nitrogen uptake by bean reduced from 14% to 50% when biochar application was increased from 30 g to 90 g per kilogram of soil.

The seedling height was measured from root collar till the tip of the vine using a plastic ruler at every three months interval. At the end of the year, maximum height (68.60 cm) was observed in 25% + compost 75% and a minimum of 7.56 cm was recorded in 100% wood biochar. It was also observed that with the increase in the quantity of biochar powder, the effect was negative. This could be due to the creation of an optimum environment like air-filled porosity, water-holding capacity and nutrient supplement ability of the medium for growth and development of the seedlings. However, when the proportion of biochar was increased, the ability of the medium to hold water increases but lacked the nutrients for the seedlings. Table 2. below shows the effect of different media mixes on seedlings girth and length.

Table 2. Effect of treatments on above-ground growth parameters of the seedlings.

Treatment	Composition	Seedling girth (mm)	Seedling height (cm)
1	Compost (100%)	5.68^{b}	41.80^{c}
2	Biochar + compost (25% : 75%)	6.69^{a}	68.60^{a}
3	Biochar + compost (50% : 50%)	5.72^{b}	41.20^{c}
4	Biochar + compost (75% : 25%)	3.53^{c}	12.23^{e}

5	Biochar (100%)	2.06[d]	7.56[f]
6	Biochar + rice husk (50% : 50%)	3.51[c]	18.50[d]
7	Biochar + vermicompost (50% : 50%)	6.40[a]	49.17[b]
	CV (%)	9.08	11.34
	P-value	0.00	0.00

Means with the same alphabets within the column are not significantly different.

3.4. Underground seedling growth

The underground parameters assessed were taproot length and number of lateral roots that had originated from the taproot. Significant effect on tap root length was observed in biochar 25% and compost 75% (44.40 cm) followed by biochar 50% and vermicompost 50% (43.20 cm). On the other hand, the least effect was recorded in 100% biochar 5 (22.13 cm).

Similarly, the taproot length significantly correlated to the number of lateral roots. This means that longer the length of the taproot higher the number of lateral roots. The highest number of laterals was seen in biochar 25% and compost 75% (25 numbers) followed by biochar 50% and vermicompost 50% (24 numbers). 100% biochar had the lowest (12 numbers). There was a significant difference in the effect on lateral roots between biochar 25% and compost 75% and treatment 7 (biochar 50% and vermicompost 50%) with that of 100% compost. However, there was no large difference in the means among the roots and shoots parameters. This may be because biochar held maximum water during irrigation which affected the root growth. The is partly substantiated by Major (2010) who explained that biochar holds a significant amount of water. Manickam et al. (2015) also reported that the addition of 4% to 5% of biochar in sandy soil resulted in a significant increase in plant-available water content from 5% to 8%.

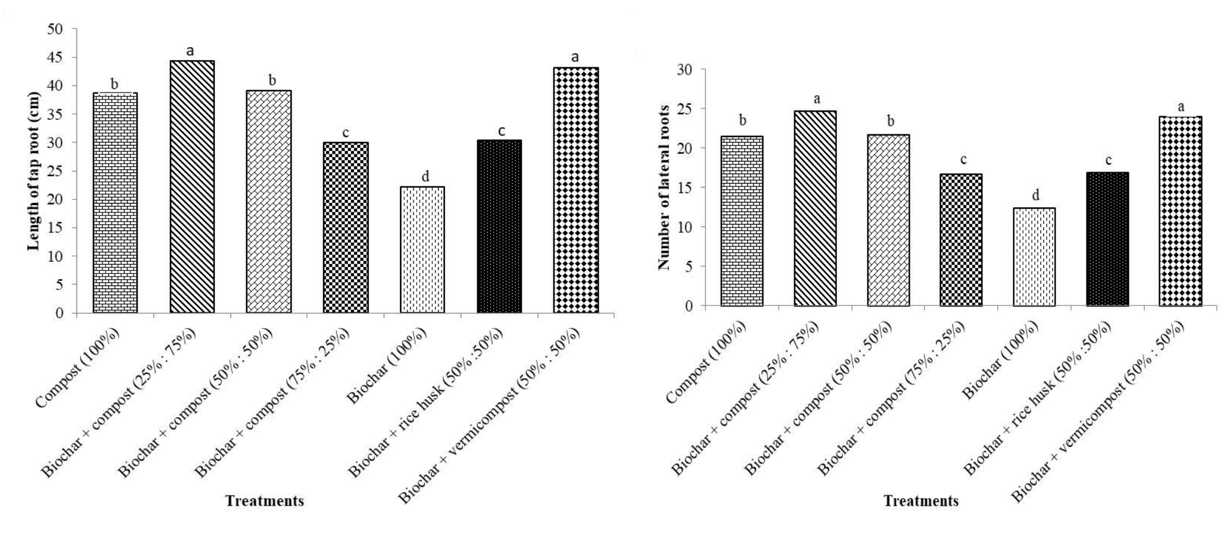

Figure 3. Effect of treatment on underground growth parameter.

On the other hand, unlike other organic soil amendments, wood biochar contained minimal plant nutrients which affected the seedling development. Also, bigger particle size-biochar derived from wood have larger pores retaining excessive water. A study by Sorrenti, Muzzi, and Toselli (2019) reported that biochar amendment influenced peach root physiology rather than its biomass. Likewise, in another report by Bruun et al. (2014), barley root density was found to have been significantly influenced with 2% straw biochar addition than by 4%.

4. Conclusion

Although biochar application has been considered to improve soil physico-chemical properties and enhance soil fertility, increase in the percentage of biochar in the growing medium did not show a positive effect in this study. Additionally, biochar proportionately increases the pH of the medium affecting the availability of nutrients to the seedlings. Wood biochar production and use can be a renewed organic soil management practice for poor quality and low pH soils. Pyrolysis without proper control of temperature yields poor quality biochar. To improve the quality of biochar, we need to have better equipment and enhanced skills. To help better control pyrolysis temperature, we need to develop or introduce kiln or huge chamber for mass production with temperature control functions. Further, we also recommend an in-depth study to assess the quality of biochar from different feedstocks and their application effects on different soil types. There is also the requirement of numerous long-term studies to draw clear conclusion regarding the effect of biochar usage on carbon sequestration potential in our context.

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Performance of Agricultural Farmers' Groups and Cooperatives in Eastern Bhutan

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ABSTRACT

The number of farmers' groups (FGs) and cooperatives (Coops) increase annually in Bhutan. However, studies on their performance are limited. Thus, this study (1) examined the performance of agricultural FGs and Coops and (2) determined the association between the performance of FGs and Coops and their selected characteristics in six districts of Eastern Bhutan. The results were generated from the data collected through group interviews of 60 groups (53 FGs and 7 Coops) in early 2020. The cooperative performance index was used to divide groups into three performance levels: (1) early transition to growth, (2) mid-transition to growth, and (3) model groups. The results showed that most groups (62%) were in mid-transition to growth, followed by the early transition to growth and model groups with 20% and 18%, respectively. FGs and Coops performed weakly in areas of management, marketing, and retention of members. The frequency of meetings and the number of literate members were positively correlated with the groups' performance. Overall, this study suggests the requirement of supports in areas of initial institutionalisation of groups, management, marketing, and product diversification to strengthen existing FGs and Coops.

Keywords: *Agriculture; Cooperatives; Eastern Bhutan; Farmers' group; Performance*

1. Introduction

There is no standard definition for farmers' groups (FGs) and Cooperatives (Coops). However, in Bhutan, the FG can be defined as "a group of not less than three members deriving economic benefits from one or more economic enterprises related to Renewable Natural Resource Sector" (*The Cooperative (Amendment) Act, 2009*, 2009). The Cooperative (Amendment) Act of Bhutan 2009 also defined Coop as "an association of persons united voluntarily to meet their economic needs through a jointly-owned and effectively governed enterprise". FGs and Coops operate based on seven principles: open and voluntary membership (1), democratic control (2), members' economic participation (3), autonomy and independence (4), education, training, and information (5), cooperation among cooperatives (6), and concern for the community (7) (ICA, 2018). Agricultural FGs and Coops are one among many other types of groups, including but not limited to consumer, credit, housings, worker, health, and social care groups (Birchall, 2004). In this study,

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agricultural FGs and Coops refer to those groups engaged in agriculture, livestock, and forestry-related activities.

FGs and Coops offer farmers with advantages that are difficult to achieve by working individually. According to Van Dijk, Sergaki, and Baourakis (2019), FGs and Coops support smallholder farmers by improving their countervailing power, transaction costs, market access, market transparency, risk management, economic of scale, professionalism, chain development, and communal interest. Although FGs and Coops are primarily formed to benefit their members, they also benefit the broader community. For example, FGs and Coops provide employment (Wanyama, Develtere, & Pollet, 2008) and improve social capital in the community (Tenzin & Natsuda, 2016). FGs and Coops are also known for reducing poverty at the country level (Tenzin, Otsuka, & Natsuda, 2015). Recognizing the myriad benefits of collective actions, the Royal Government of Bhutan (RGoB) has been promoting FGs and Coops in Bhutan.

Bhutan occupies and area of 38,394 square kilometres; and a population of 681,720, of which 66% live in rural areas (NSB, 2019). Although cultivated land is only about 3%, agriculture is one of Bhutan's five economic jewels (NSB, 2019). The agriculture sector employed 53.9% of Bhutanese and contributed 17.37% to Bhutan's gross domestic product (NSB, 2019). Bhutanese farmers mostly practice self-subsistence integrated farming, comprising crops, livestock, and forestry (GNHC, 2017; Sonam & Martwanna, 2012). In the 9th Five-Year Plan (2002-2007) and 10th Five-Year Plan (2008-2013), the RGoB promoted FGs and Coops to commercialize self-subsistence agriculture by strengthening production and marketing (GNHC, 2008; Sonam & Martwanna, 2011). This study uses the word 'groups' interchangeably with FGs and Coops for convenience.

In 2009, the Ministry of Agriculture and Forests (MoAF) created the Department of Agricultural Marketing and Cooperatives (DAMC). The DAMC functions as a full-fledged departmental agency under the MoAF to strengthen marketing structures, institutional linkages, and FGs and Coops (DAMC, 2019a). As of June 2019, the DAMC registered 509 FGs and 71 Coops in Bhutan (DAMC, 2019b). The number of registered FGs and Coops in Bhutan has drastically increased over the years. Besides, many informal groups remain un-registered with the DAMC, which will increase the number of registered FGs and Coops in the country shortly.

While it is acceptable to increase the number of FGs and Coops in the country, it also equally essential to have better-performing groups to deliver the expected benefits. Thus, assessing the performance of FGs and Coops is of paramount importance. Previous studies used different models to measure the performance of FGs and Coops. Some related studies used financial models, such as cost, profit, sales growth, returns on assets, returns on equity, and returns on sales (Hartikayanti & Permadhy, 2015; Khan, Yaacob, Abdullah, & Abu Bakar Ah, 2016; Mishra, Wilson, & Williams, 2009). However, non-profit oriented FGs and Coops do not prioritize their goals to financial growth (Mayo, 2011). Thus, some studies used non-financial models such as members' commitment, trust, or satisfaction to measure the performance (Österberg & Nilsson, 2009; Torres-

Lara, 2000). Some studies also combined financial and non-financial models (Eriksson & Li, 2012; Govori, 2013). In recent years, more holistic models were deployed to measure FGs and Coops' performance using multiple indicators (Masango, 2015; Nkuranga & Wilcox, 2013). Considering the inclusion of diverse FGs and Coops in this study, we also partially adopted a holistic model from Nkuranga and Wilcox (2013).

Improving FGs and Coops' performance requires a comprehensive understanding of knowledge affecting the performance of these groups. The resource-based view theory, the basis of this study, implies that the organisations have resources necessary for influencing its performance. This theory suggests that the organisations having valuable, rare, inimitable, and non-substitutable resources (internal factors) will perform better (Barney, 1991). Previous studies show internal factors that significantly affect the performance of FGs and Coops. Internal factors include chairperson's age (Kanfer & Ackerman, 2004), number of years as chairperson (Purves, Niblock, & Sloan, 2015), operating years (Barham & Chitemi, 2009), board sizes (Bond, 2009), frequency of meeting (Ruengdet & Wongsurawat, 2010), number of literate members (Hasan & Almubarak, 2016), and total members (Thaba, Anim, & Tshikororo, 2016).

However, not much is known about these groups' performance and the factors affecting their performance, especially in Eastern Bhutan. Filling up this study gap will shed new lights on the performance of groups in the Bhutanese context, which would help stakeholders design programs to strengthen FGs and Coops in Eastern Bhutan. Thus, this study assessed the performance of agricultural FGs and Coops (1) and determined the association between the performance of FGs and Coops and their selected characteristics (2) in the six districts of Eastern Bhutan.

2. Methodology

2.1. Study area

Bhutan can be divided into four regions: East, West, Central, and South. This research was conducted in six districts of Eastern Bhutan: Lhuentse, Mongar, Samdrup Jongkhar, Pema Gatshel, Trashigang, and Trashi Yangtse (Figure 1). Eastern Bhutan was chosen due to the general absence of research to underscore the FGs and Coops' performance in the region. There are few related articles, but they are limited to West and Central Bhutan focusing on dairy groups (Sonam & Martwanna, 2012; Wangchuk et al., 2019). This study gap encouraged the authors to conduct this research in Eastern Bhutan.

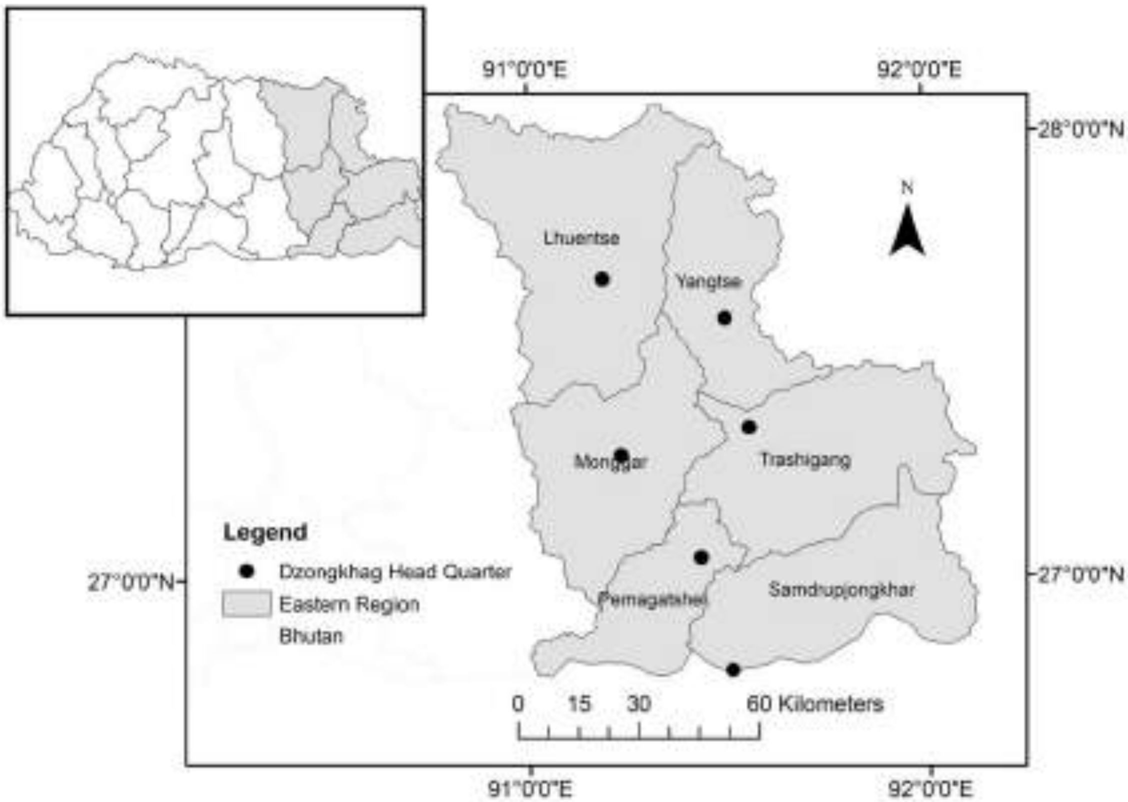

Figure 1. The study area (six districts of Eastern Bhutan).

2.2. Sample size and sampling

The target population for this study was 211 groups from Eastern Bhutan (DAMC, 2019b). The study initially planned to collect data from 74 groups (60 FGs via proportionate random sampling and 14 Coops via census). However, we surveyed 62 groups only (55 FGs and 7 Coops) as the RGoB imposed Covid-19 travel restriction at the later stage of the data collection. Further, we excluded two non-functional groups as they could not respond to most of the questions. The groups in eastern Bhutan are mostly similar in market size, organisational existence, and functioning. Therefore, this study considered data from 60 FGs and Coops from the eastern part of Bhutan (Table 1).

Table 1. Sampling of FGs and Coops.

Dzongkhag	FGs	Coops	Total
	TP(PS)[AS]	TP(PS)[AS]	TP(PS)[AS]
Lhuentse	11 (3) [3]	0 (0) [0]	11 (3) [3]
Mongar	70 (21) [20]	4 (4) [4]	74 (25) [22]
Trashigang	48 (15) [16]	3 (3) [2]	51 (18) [18]
Trashi Yangtse	23 (7) [7]	1 (1) [1]	24 (8) [8]
PemaGatshel	20 (6) [6]	0 (0) [0]	20 (6) [6]

Samdrup Jongkhar	25 (8) [5]	6 (6) [2]	31 (14) [5]
Total	197 (60) [55 *]	14 (14) [7]	211 (74) [62]

*Two FGs excluded

TP(PS)[AS] = Total population (Planned sample size) [Actual sample size]

2.3. Data collection

The primary data collection approach was group interviews (conducted in early 2020); thus, an individual FG and Coop served as a study unit. The researchers, accompanied by representatives from the Regional Agricultural Marketing and Cooperative (RAMCO), Mongar, facilitated the group interviews. Chairpersons, other position holders in groups such as accountants and treasurers, and group members participated in the group interviews. The participants responded to the questionnaire upon finalising the answers in the group.

The questionnaire was prepared in consultation with the officials from the RAMCO, Mongar, as the institution looks after the FGs and Coops in Eastern Bhutan. The questionnaire was semi-structured, and it consisted of three parts. The first part collected data on the profile of the groups and the process of group formation. The second part underscored the data on independent factors that influenced the performance of groups. The third part gathered data to measure the performance of groups. The questionnaire was pre-tested with three FGs before the actual data collection and amended where necessary for clarity. We also referred to published articles, office documents, DAMC online database, and documents from districts and gewogs. Moreover, informal interviews and field visits gave insights useful for corroborating the results from different data sources.

2.4. Data analysis

Microsoft Excel 2010 was used for data entry and cleaning. The Statistical Package for the Social Sciences (SPSS) version 25was used to analyse the data. Groups' performance was measured using the cooperative performance index (CPI) model, which was developed, tested, and adopted by the United States Agency for International Development. The CPI is based upon five areas: governing, planning, accounting, producing, and marketing (Nkuranga & Wilcox, 2013). FGs and Coops rated 54 indicators (List 1) of CPI as 1 (if they agree) or 0 (if they disagree). The CPI scores guided the grouping of FGs and Coops into three categories:(1) the early transition to growth (CPI scores less than 50%),(2) the mid-transition to growth (CPI score between 50% and 70%), and (3) the model groups (CPI scores above 70%).Spearman's correlations were also conducted to determine the correlation between the selected profiles of groups and groups' performance. The results are presented in the form of Tables and Figures.

List 1. Indicators adopted under five dimensions of CPI (Nkuranga & Wilcox, 2013).
First Dimension. Legal Status & Cooperative Planning and Strategy

1. *Does the FG/Coop have a copy of by-laws?*

2. *Are board members trained in record and bookkeeping?*
3. *Does FG/Coop prepare financial reports?*
4. *Do all FG/Coop board members participate in board meetings?*
5. *Does AG/Coop follow the by-laws?*
6. *Does the FG/Coop have all copy of regularly scheduled meetings?*
7. *Does the FG/Coop have a short-term action plan?*
8. *Do board members keep the minutes of the meetings?*
9. *Does the FG/Coop follow up the minutes of the meetings?*
10. *Does the FG/Coop have a long-term business plan?*
11. *Does the FG/Coop follow the business plan?*
12. *Are action plans voted during the General Assembly?*

Second Dimension. Management Structure and Accounting System

1. *Have members ever changed inputs applied to improve production?*
2. *Are grievances and conflict resolution procedures in place?*
3. *Do members use grievances mechanism?*
4. *Does the FG/Coop have all the necessary books of accounts?*
5. *In the past two years, did AC/FG determine if it is profitable?*
6. *Are financial reports published and shared with members?*
7. *Has the FG/Coop communicated its surplus to members?*
8. *Does FG/Coop have documented financial procedures?*
9. *Does general assembly decide on the use of the surplus?*
10. *Do employees have clear job descriptions and contracts?*
11. *Are members of the supervisory committee trained in FG/Coop supervision?*
12. *Does FG/Coop have a paid accountant?*
13. *Does the FG/Coop have any other paid staff?*
14. *Does staff have performance agreements with the FG/Coop?*
15. *Does FG/Coop conduct regular internal audits?*
16. *Is financial reporting is timely audited?*
17. *Does FG/Coop have an operational manager?*
18. *Does a computerised accounting system exist?*
19. *Does FG/Coop conduct regular external audits?*
20. *Performance evaluations guide the incentives*.

Third Dimension. Production and Quality of Inputs

1. *Does FG/Coop train and provide technical support on improved production methods?*
2. *Did the FG/Coop production increase due to market demand?*
3. *Does your FG/Coop production satisfy the market needs?*
4. *Is there regularly quality assurance checking of inputs used by executives?*
5. *Does the FG/Coop do market-led production?*
6. *Does FG/Coop add value to members' production?*
7. *Does your FG/Coop update its market study to meet the client's expectations?*

8. *Does the FG/Coop collect and markets products on behalf of its members?*
9. *Does FG/Coop have the capacity to bulk and distribute farming inputs to members?*

Fourth Dimension. Market Linkages and Business Relations

1. *Does the FG/Coop provide market information to its members?*
2. *Does your FG/Coop have a market plan?*
3. *Does your FG/Coop have a contract with clients?*
4. *Does the FG/Coop have a marketing committee?*
5. *Has your FG/Coop developed marketing materials, business cards, name plaque for exhibitions, office, signposts, etc.?*

Fifth Dimension. Recruitment and Member Retention Strategy

1. *Are there clearly defined membership criteria?*
2. *Have all members paid the membership fees (Share capital)?*
3. *Are member needs integrated into the planning process?*
4. *Is there a regular survey of members' needs?*
5. *Does FG/Coop have a record of member activities?*
6. *Do FG/Coop member-trainers train other members?*
7. *Does FG/Coop provide a dividend to its members?*
8. *Does FG/Coop have a training fund?*

2.5. Ethical considerations

The Research Committee of the College of Natural Resources approved the research proposal. The RAMCO office also sent an official letter to the DAMC, districts, and gewogs informing about the research and soliciting their corporations during data collection. The enumerators explained the objectives of the study and assured the confidentiality of the information to the participants. The participants could withdraw from the study if they were not interested. Verbal consent was obtained from all the participants before the commencement of the data collection.

3. Results and Discussion

3.1. Profile of FGs and Coops

About 72% of FGs and Coops in Eastern Bhutan are in the production sector. Potential exists in the promotion and integration of service and processing sectors. Fifty per cent of the groups reported a decline in the number of members than their initially registered members. The result showed that most groups had difficulty in retaining their members. Most groups (60%) chose men as their chairperson. O'Brien and Wegren (2015) also reported such under-representation of women leaders in collective actions. The low representation of women in a leadership position is likely, as men leaders tend to outperform women in improving their organisation (Barham & Chitemi, 2009). Thus, people appear to trust men compared to women as leaders. Most groups (80%) had elected literate individuals as their chairperson. Educated leaders are directly linked with the success of groups (Gutema, 2014; Nyoro & Ngugi, 2007), suggesting that members of the FGs and Coops considered qualifications in choosing their leaders.

About 80% of the groups have reported that the ideas proposed by the gewog (sub-district), district, or RAMCO have led to the formation of groups. Sonam and Martwanna (2012) also reported that the formation of groups in Bhutan is a mostly top-down approach. The top-down approach decreases the sense of ownership (Sonam & Martwanna, 2012; Vilas Boas & Goldey, 2001). Contrarily, groups succeed when ideas originate from their members (Pathak & Kumar, 2008). Thus, Van der Walt (2005) suggests that the initial idea to form the group must come from the group's members. Table 2 presents the selected profiles of the FGs and Coops in Eastern Bhutan.

Table 2. Profile of FGs and Coops in Eastern Bhutan.

Variables	Categories	Frequency	Percentage
Business idea	Members	12	20.0
	Gewog, Dzongkhag, or RAMCO	48	80.0
Business sector	Agriculture	38	63.3
	Livestock	22	36.7
Nature of activities	Production	43	71.7
	Processing	16	26.7
	Service	1	01.7
Member size changes	Decreased	30	50.0
	No Change	14	23.3
	Increased	16	26.7
Gender of chair	Male	36	60.0
	Female	24	40.0
Qualification of chair	None	12	20.0
	Non-Formal Education	18	30.0
	Class PP to VI	14	23.3
	Class VII to X	10	16.7
	Class XI to XII	6	10.0

Note: Non-Formal Education includes monastic education, and PP refers to pre-primary schooling.

3.2. Performance of FGs and Coops

About 20% of the groups were in the early transition to growth stage. These were nascent FGs and Coops that needed extra support for survival and growth. This category of the group had very few or no activities. Besides, the FGs and Coops had poorly developed by-laws, weak management, attrition of member, and negligible benefits to the members. Such groups are likely to dissolve in the absence of timely and effective interventions. Thus, stakeholders should strive to make these groups functional through proper institutionalisation. The focus should be on team building, visualising the goals, and revising the group by-laws.

Most FGs (62%) were in the mid-transition to growth stage. The performance of these groups was moderate - at the growing stage. Since these groups were already operational, they had a few regular activities. These groups benefited their members in cash and kind. Sometimes, they also exhibited their spill-over benefits to communities. As they were operational, their significant challenges were the management of groups and market access. Thus, interventions were required for strengthening groups and improving market access. Wangchuk et al. (2019) and Sonam and Martwanna (2012) had suggested similar recommendations. These groups were small scale dealers, depending on external support for their survival.

The model groups comprised only 18% of the total FGs and Coops. The best FG in 2019, the Norbugang Zambala Dairy Group (Rabten, 2019), was in this category. Model FGs and Coops had well-established business and regular activities. Such a stable business not only benefited its members but also generated spill-over benefits in the community. For instance, these groups employed their members and other individuals from the communities, either as full-time or part-time. However, most FGs and Coops in Bhutan are mostly primary producers; therefore, they should explore the processing and value addition; and promote their products in export markets.

3.3. Comparing Performance among Five Dimensions of CPI

The dimension “Production and Quality of Inputs” scored the highest. This dimension had nine indicators to assess the production and input performance of FGs and Coops. This dimension scoring the highest is plausible because 71% of the groups were primary producers focusing on input quality and increasing production volume. The Agriculture Research and Development Centre (ARDCs) and stakeholders also provide groups with technical supports.

The dimension "Legal Status and Cooperative Planning and Strategy" comprised 12 indicators. These indicators assessed the groups’ performance on legal and planning aspects. This dimension included statements like “does the FG or Coop have a copy of by-laws?” This dimension had the second-highest average score of 42. Registration of FGs and Coops in Bhutan must go through three stages. Gewog initially approves the farmers’ application to form the group and then submit it to the district in the first stage. The district's focal person reviews the application and sends it to the DAMC in the second stage. The DAMC registers the groups in the third stage. Thus, the entire registration process demands planning and fulfilling legal requirements. Also, focal persons at all stages help in planning and checking legal issues (The Cooperative (Amendment) Act of Bhutan 2009, 2009). Therefore, it was reasonable for FGs and Coops to score the second-highest on this dimension. Figure 2 compares the average scores among the five dimensions of the CPI.

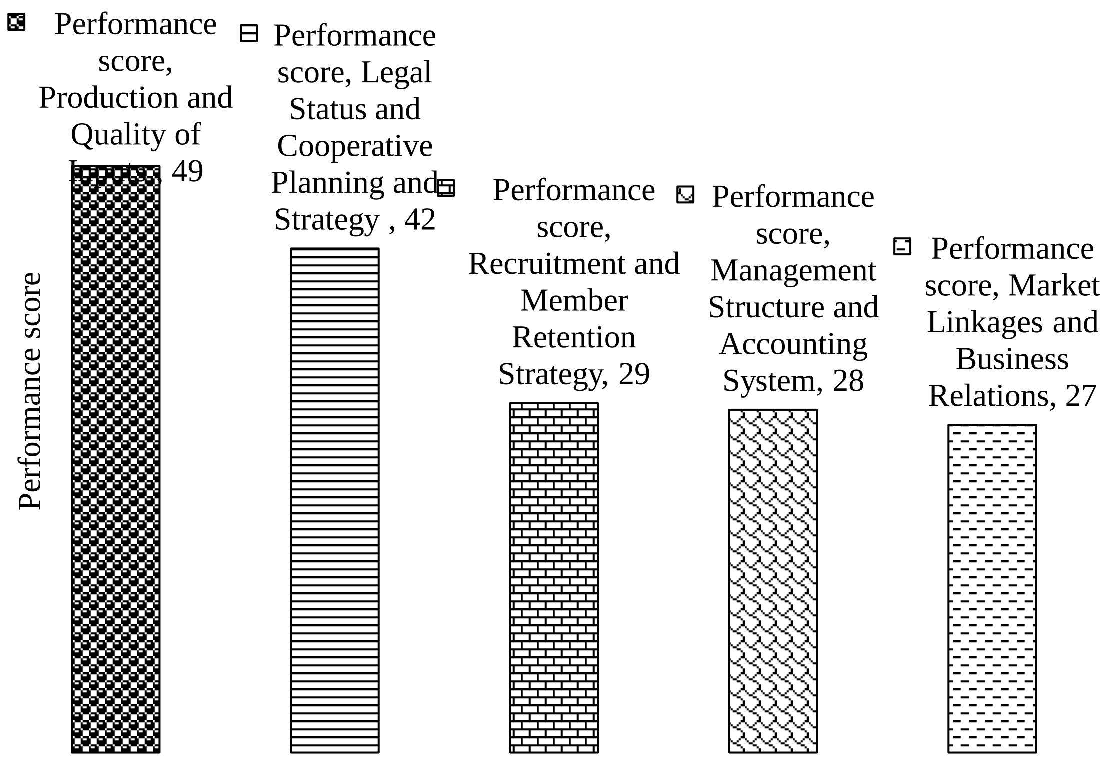

Figure 2. Comparison of performance of five dimensions of CPI.

The third, fourth, and fifth dimensions (from left to right in Figure 2) had 8, 20, and 5 indicators, respectively. On average, all three dimensions scored less than 30. Scores were lower than the two leading dimensions' scores – production and quality of inputs (1) and legal status and cooperative planning and strategy (2). Lower scoring of these three indicators shows that groups face more problems related to management, accounting, marketing, and retaining members. Our field observations had evidence that supported the existence of these issues. For instance, many groups were not able to enforce by-laws. We also noted no proper bookkeeping and auditing system. The group also faced challenges in marketing their products. Also, marketing committee, business card, signposts, and websites were not available in most groups. Wangchuk et al. (2019) and Sonam and Martwanna (2012) reported these issues in the West and Central Bhutan. Thus, promoters of FGs and Coops should focus more on improving recruitment and retention of members; management and accounting; and marketing and business relation.

3.4. Success factors of FGs and Coops in Eastern Bhutan

The second objective was to determine the association between FGs and Coops' performance and their selected characteristics. Thus, we performed the correlation between selected characteristics of groups and their performance (Table 3). Non-parametric data necessitated conducting Spearman’s correlation coefficients. The frequency of meetings correlated with the performance of groups at 0.01 level of significance. The correlation was positive and moderate (r = .355). FGs and Coops are member-oriented undertakings requiring frequent meetings. Frequent meetings enable better job divisions, monitoring, and coherence in groups. The current result agrees well with previous studies (Ruengdet & Wongsurawat, 2010; Sabatini, Modena, & Tortia, 2014; Xie, Davidson, & DaDalt, 2003). Thus, it implies the need for frequent meetings in groups for their better performance.

The number of literate members in a group significantly correlates with the group performance ($P<0.01$). The association was moderate and positive (r = .430). Certain levels of numeracy and literacy were necessary for managing groups. Several studies published that education has a role in the groups' performance (Amini & Ramezani, 2008; Barham & Chitemi, 2009; Fahlbeck, 2007; Garnevska, Liu, & Shadbolt, 2011; Hasan & Almubarak, 2016). Thus, groups should often meet to discuss, plan, and track activities.

This study also examined the correlations of the performance and five other characteristics of groups (chairperson's age, number of years as chairperson, operating years, board sizes, and total members) showing significant associations in previous studies (Barham & Chitemi, 2009; Bond, 2009; Kanfer & Ackerman, 2004; Purves et al., 2015; Thaba et al., 2016). Unlike our expectation, they did not correlate with the groups' performance in this study. It is plausible because most groups are small-scale producers' groups with no complex management structure. However, other studies support the non-significant correlation between the performance and characteristics of FGs and Coops (Azadi et al., 2010; Rabirou, Olusayo, & Okparaocha, 2013; Rajaei, Yaghoubi, & Donyaei, 2011).

Table 3. Relationship between selected characteristics and performance of FGs and Coops.

Variables	1	2	3	4	5	6	7	8
1. Performance	1							
2. Age of chair	-.021	1						
3. Years as chair	.084	-.063	1					
4. Operating years	.119	.178	.400**	1				
5. Board members	-.225	-.294*	-.127	.129	1			
6. Meeting	.355**	-.166	-.010	.358**	.204	1		
7. Literate members	.430**	-.061	-.039	-.056	-.046	.223	1	
8. Total members	-.022	-.106	-.201	-.073	.272*	.273*	.457**	1

** Correlation is significant at the .01 level (2-tailed)
* Correlation is significant at the .05 level (2-tailed)

4. Conclusion

This study has two crucial findings on FGs and Coops' performance from the eastern part of Bhutan. Most groups (62%) where in the mid-transition to growth phase, performing weakly in management, marketing, and retaining members. The frequency of meetings and the number of literate members in the group correlate with the group's performance. Thus, groups in different performance categories require specific supports. The nascent groups needed proper institutionalisation, including team building, visualising goals, and revisiting by-laws. We recommend stakeholders intervention in management and marketing areas for the groups in mid-transition to the growth phase. The matured groups needed support in product diversification through processing and value addition. An opportunity exists for the model groups to explore their products in international markets. The study also highlights the importance of regular meetings and literate members for better performance of the groups.

This study's limitation is that results are solely based on the CPI model used for measuring the performance of groups; thus, future researchers could use other models to compare the current results.

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High Yielding Indeterminate Bean Varieties to Diversify Bean Farming

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ABSTRACT

Common bean is an important vegetable which provides carbohydrates, proteins, vitamins and minerals for human nutrition, and is often used as a meat alternative in poor countries. Demand for beans for the domestic market in Bhutan is huge which results in increased imports annually. The ban on bean imports citing pesticide residues further led to imbalance in the market (supply-demand) equilibrium. To diversify the choice of cultivars for increased production and to help stabilize market imbalance, two improved cultivars from Japan were evaluated for yield stability, yield potential, length of growing season, pest/disease tolerance, and market and agronomic traits. Grey pole bean was used as a check variety for the study. The variety Prime Green, followed by Brown Pole is preferred over Grey Pole on a range of crop characteristics such as yield potential, disease/pest tolerance, marketable traits (fibrous [stringy] and fleshiness of the pods), and agronomic traits (germination rate, and days to maturity). All three cultivars evaluated show yield stability.

Keywords: *Indeterminate beans; Stable yield; Agronomic traits, Market traits*

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is the second important legume grown for consumption next to Faba bean (*Vicia faba* L.), with 90 % of the area under cultivation (Celmeli et al., 2018). It constitutes more than half the grain legume consumed in the world (Broughton et al., 2003). It is one of the important vegetable crops in world known for its great adaptability and diversity (Vidak et al, 2015 as cited in Tenzin, Lhadon, Phuntsho, & Lhadon, 2018).

Common beans contain carbohydrates, protein, and important vitamins and minerals and are widely used as an alternative protein source to meat, egg and dairy products (Blair et al, 2003 as cited in Tenzin et al., 2018). With the existing poor dietary habits of Bhutanese, wherein carbohydrates rich food, but poor in protein (Tenzin et al., 2018) are predominant, beans are perfect functional food for human nutrition (Câmara, Urrea, & Schlegel, 2013).

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Domestic production of beans doubled in 2018 from 2014 and recorded a concurrent rise in annual import (RSD, 2017) until bean import was banned in 2016 citing the presence of chemical/pesticide residues (DAMC, 2017). In Bhutan, vegetables including beans are cultivated on 29% of the arable land (Katwal, 2013), making it the fifth and sixth most important vegetable crop by cultivation area and production, respectively. The import ban has further aggravated the already imbalanced domestic market equilibrium resulting from land-use conflicts, pests, and human-wildlife conflicts besides others (Katwal, 2013).

Although a diverse range of bean landraces are reported grown widely under different agro-ecological zones in eastern Bhutan (Tenzin et al., 2018), only a few indeterminate genotypes yielded high. Bhutan has only two indeterminate improved varieties registered for cultivation (DoA, 2019) registered, the yields are comparatively lower than indeterminate cultivars (ARDC-Bajo, 2020).

Thus, bean production in Bhutan is limited to a few high yielding cultivars and constrained by land-use conflicts in addition to several other challenges. Therefore, there is a need to diversify improved varieties in the country that would provide higher net returns out of limited resources, and to help alleviate demand gaps through increased production. This study was aimed at evaluating the performance of two indeterminate high yielding bean varieties under Bhutanese conditions.

2. Materials and methods

2.1. Planting material

The varieties were introduced from Japan in collaboration with the Integrated Horticulture Promotion Project (IHPP).

2.2. Location and time Period

The experiment was conducted on-station at ARDC-Bajo under researcher managed experimental trial for two consecutive years (2018-2019). The following year, trials were conducted at three locations at different altitudes in west-central Bhutan under farmers management conditions. (Table 1).

Table 1. Location of the varietal trial.

Location	**Dzongkhag (District)**	**Altitude(*masl*)**
ARDC Bajo (on-station)	Wangdue	1210
Tsendagang	Dagana	650
Rubesa	Wangdue	1350
Limbukha	Punakha	2200

2.3. Experiment Design

Two indeterminate bean varieties viz., Prime Green and Brown Pole were evaluated with Grey Pole as the check variety. The experiment was laid out using a Randomized Complete Block Design (RCBD) with four replications. During the field preparation, 3t/acre compost and 0.5t/acre Suphala (NPK=15:15:15) were applied as basal application. One month after germination, 0.5 t/acre Suphala was top-dressed uniformly (Tomiyasu et al., 2018). Spot sowing of seeds at 25cm between the plants and 30cm between the rows was carried out on 2nd March at ARDC Bajo farm field (on-station). Similarly, beans were grown during their normal growing season at three different places in the farmer's field. Seeds sowing were carried out on 6th march at Tsendagang under Dagana, 15th march at Rubesa under Wangduephodrang Dzongkhags, and on 4th April at Limbukha under Punakha Dzongkhag. On-farm trial plots were subjected to farmer's management practices, except the uniform plant spacing that was followed the same throughout the trial plots.

2.4. Data collection and Data analysis

Bean pods were harvested seven times at horticultural maturity. The bright green, fleshy and tender pods were harvested over a month from 28th April until June last week at ARDC-Bajo (on-station). Similarly, first fruit harvesting commenced from the first week of May at Rubesa and Tsendagang under Wangduephodrang and Dagana dzongkhags, respectively, and from the first week of June at Limukha under Punakha Dzongkhag. Yield data was collected from 7m^2 treatment plots from both on-station as well as from the on-farm trial plots. Quality attributes such as pod weight, pod diameter, pod length, seed count per pod, number of pods per plant, days to maturity, and the germination rate of different varieties were recorded. Incidences of insect pests and diseases were recorded as percent pod infested, while diseases were recorded on a scale of 1 – 4 (Manandhar et al., 2016).

Preferences for the cultivars by collaborating farmers were studied using a semi-structured questionnaire. Information of yield, disease and pest resistance, market demand, early maturity, pod texture, string and string-less nature of pods were assessed on a scale of 1-5 (where; 1=not good, 2=good, 3=moderate, 4=very good, 5= outstanding). Data were analyzed using Statistical Tool for Agricultural Research (STAR) version 2.0.1and MS Excel 2019. Mean yield, and quality attributes were subjected to one-way ANOVA in STAR 2.0.1.

3. Results and Discussion

3.1. Agronomic traits

Yield potential and stability

Significant yield differences *($P< 0.05$)* were observed among the treatments at on-station as well as on-farm plots (Table 3). No significant differences were observed in the yield of varieties grown under different locations cultivated in normal growing seasons of the chosen localities (Table 2). There is significant interaction between years and treatments in the yield of the beans.

Table 2. Summary of combined analysis.

SOV	SS	DF	MS	F-cal	F-tab		*p* value
					0.05	0.01	(α=5%)
Years	10400.01	1	10400.01	2.57776	5.99	13.75	
Replication*Years	24207.03	6	4034.51				
Treatment	83312.9475	2	41656.5	22.413**	3.9	6.93	0.000089**
Years*Treatment	11384.1375	2	5692.07	5.59565*	2.85	4.5	0.019193*
E_{pooled}	22303.1	12	1858.59				
Total	151607.225	23					

Table 3. Yield differences among the varieties at ARDC Farm field (on-station).

Variety	**2018 (t/ac)**	**2019 (t/ac)**
Grey Pole (check variety)	4.6 b	4.8 b
Prime Green	7 a	7.3a
Brown Pole	4.5 b	4.7 b
P- value	0.02	0.004
CV%	9.8	13.87

Under all the experimental plots, Prime Green (a.k.a. White Pole) out yielded brown pole and grey pole varieties (Table 3). The yield of the crop is largely attributed to the use of different cultivars, besides other factors such as biotic (pest and disease), abiotic (climatic factors, soil fertility, topography, and water), and the technological factors (Grassbaugh & Bennett, 1998). It was also pointed out that plant attains the highest potential yield under congenial growing conditions (Decoteau, 1998).

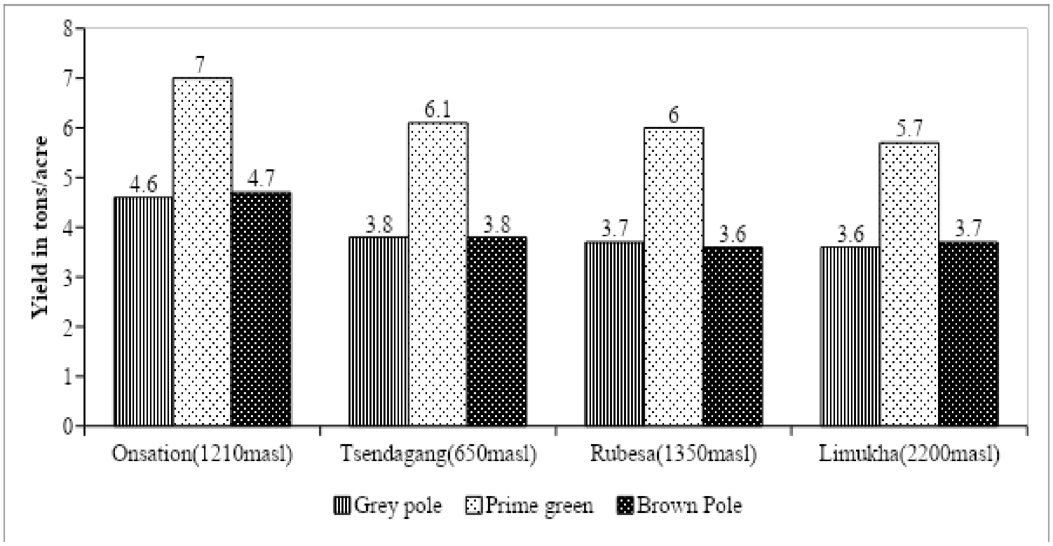

Figure 1. On-station and On-farm Yield comparison.

Yield attributes

The quality attributes of the test varieties such as pod count per plant, weight of the individual pod, pod length and pod diameter were measured (Table 4). Prime Green records highest pod count per plant, and highest pod length than Brown Pole and the check variety. For all these test varieties, pod diameter and seed count per plant almost remained the same.

Table 4. Quality attributes of the test varieties.

Treatment	**Pods/Plant**	**Pod weight (cm)**	**Pod length (cm)**	**Pod diameter (cm)**	**Seeds/Pod**	**Days to Maturity**	**Desirable trait**
Grey Pole	43.5	11.1	14.3 b	1.07	8a	61a	Stringed
Prime green	55	10.9	16 a	1.05	8a	61a	Stringless
Brown Pole	47	12.4	14.4 b	1.25	7b	56b	Mildly stringed
P- value	0.34	0.26	0.017	0.09	0.26	0.0001	
Mean	48.42	11.49	14.8	1.09	7.75	64	
CV (%)	21.5	10.88	3.61	5.06	3.72	0.9	

*****Means with the same letters in a column are not significantly different at 95% confidence level.

Full bloom and days to maturity

The varieties Prime Green and Grey Pole (check variety) took 46 days to reach its full bloom, whereas the variety Brown Pole saw its full bloom at 41 days after sowing. Brown Pole attained horticultural maturity in 50-57 days after sowing, while Prime Green and Grey Pole took 58-64 days. Thus, the variety Brown Pole has the scope to market 7-8 days earlier than Prime Green and Grey Pole. Further, the variety Brown Pole has the potential to market earlier than the already existing varieties like White no. 1by 13-15 days (DoA, 2019). Hence, the variety Brown pole was found as early maturing variety among the three varieties evaluated.

Germination

No significant difference (*P-value < 0.05)* was observed in the germination percentage of test varieties. All the tested varieties showed a germination rate between 90-95 %. The seed germination, however, is solely dependent on the prevalent soil moisture and soil temperature during the seed sowing time. It is observed that seeds germinate at optimum soil moisture, temperature and oxygen (DuPont, 2012). Similar findings were reported where genotypes of *Phaseolus vulgaris* L. exhibited an increased rate of the seed germination from 8-29 °C (White & Montes - R, 1993), and 94% germination rate was recorded at an optimum temperature of 22°C (ARDC-Bajo, 2020; Nleya, Ball, & Vandenberg, 2005). ARDC-Bajo (2020) also recommends beans seeding at an average temperature of 18-27°C at 60-70 % relative humidity.

Major Disease and Pest

Leaf rust incidence was observed less than 5 % on Prime Green and 10-15% on Brown Pole varieties under normal growing conditions. Disease severity of 2 on Prime Green, and 4 on Brown Pole and Grey Pole on a scale of 1-4 was recorded.

Although no major insect pest occurrence was observed, minor incidences of pod borer infestation were observed in all the varieties. No significant difference was observed in insect pest infestation among the varieties. During the two years study period, it was observed that less than 2% of the pods were infested with pod borer. Moderate pod borer incidence of 31 % in common bean (Karel, 1985), 54 % in cowpea and 24-40 % in pigeon pea (Sharma, Saxena, & Bhagwat, 1999) were observed in other countries., However, Dorji, Dorji, and Fujiie (2019) reported 8-10 % of the incidence in the west-central region of the country.

3.2. Market traits

Farmers chose the two tested varieties Prime Green and Brown Pole over check variety Grey pole (Table 5). Characteristics such as soft and fleshy pods, string-less, high market demand, and high yielding favored its assessment over the two.

Although mildly stringed with fleshy and soft pods, variety Brown Pole matures comparatively earlier than already registered bean varieties maturing by 13-15 days (DoA, 2019), and by 7-8 days earlier than other two tested variety.

Table 5. Variety preference rank.

Variety	Ta	HY	MD	Ma	Disease	Pest	Easy cooking	Fleshy	String less	Rank
Grey Pole	3	3	2	3	3	3	3	3	2	**3**
Prime green	4	5	5	4	4	4	5	5	5	**1**
Brown Pole	4	3	3	5	3	4	3	3	3	**2**

*Ta-taste, HY-High yielding, MD-Market demand, Ma- Early maturity, Pest- Pest tolerance, Disease-Disease tolerance.

4. Conclusion

The tested variety Prime Green with its potential to yield 7 t/acre on average out yielded varieties Brown Pole and Grey Pole bean which obtained an average yield of 4.7 and 4.6 tons per acre, respectively. Soft and fleshy pods without fibrous strings are attributed for its high market demand and thus farmers ranked it as their preferred variety followed by the variety Brown Pole. Promotion of these two varieties will increase the productivity as well as varietal diversity of common beans in Bhutan.

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Agronomic Parameters of High-Altitude Rice Varieties and their Relation to Temperature at Different Growth Stages

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ABSTRACT

The warm temperate agro-ecozone or high-altitude rice production areas in Bhutan represent a unique agro-ecology that is specific to adaptation needs of rice varieties. A study was undertaken to consolidate the basic agronomic parameters released and popularly cultivated rice varieties of the high-altitude areas. The agronomic parameters were assessed in relation to the weather in the zone. Ten years' weather data (2009-2018) of Paro Dzongkhag was used to relate the agronomic parameters of rice varieties to temperature and rainfall patterns. Seven improved varieties and one traditional variety were used for this study which was conducted at Tsento-Shari ((2450 masl), Paro in 2019 rice season. 50% flowering and days to maturity which are the two most fundamental indicators of adaptation of rice varieties in the warm temperate agro-ecozone were at 162-181 days and 206-236 days respectively. The mean temperatures in the trial site for 10 years during anthesis was 18.5 ºC against the optimum mean temperature requirement for rice (30-33 ºC). The mean temperature at the ripening phase was found to be 16.5 ºC as against the optimum temperature requirement of 20-25 ºC. Despite a relatively low mean temperature during ripening, all the assessed varieties matured and produced good yield indicating good cold tolerance. Rice farmers in the high-altitude areas normally transplant seedlings at about 90 days old when they are hardy enough and can gain some level of physiological maturity to help cope against low mean temperature at ripening. The mean yield of the eight varieties ranged from 2.01 t/acre to 2.98 t/acre which was higher compared to the national average yield of 1.73 t/acre.

Keywords: *Warm Temperate agro-ecozone; Agronomic parameters; 50% Flowering; Days to Maturity; Temperature; Rainfall*

1. Introduction

Rice is the most important crop in the world as more than half of the world's population depend on it as the staple food (IRRI, 2020). Bandumula (2018) reported that eleven countries of Asia contribute about 87% of the global rice production and hence Asia significantly contributes in achieving global food security. However, the rice sector is hit by many abiotic, biotic and socio-economic constraints that hinder its production (Fahad et al., 2019). Among biotic stresses, Sridevi and Chellamuthu (2015) revealed that weather is the most important determinant of growth, yield and production of rice. These challenges are further exacerbated by the decreasing number of rice

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farmers and a fast-growing global population, making it more difficult to meet increasing rice demands (IRRI, 2020). Though the rice area and production in Bhutan is meagre in comparison to the rest of the Asian countries, rice is an indispensable part of the Bhutanese diet as it constitutes a large portion of our daily calorie. Moreover, the majority of the Bhutanese population (57%) depends on agriculture for their livelihood (MoLHR, 2016). Due to the pivotal role it plays in our economy and livelihood, rice is also considered synonymous to the food security of our nation (Chhogyel & Bajgai, 2015). The annual production of rice in 2018 was 63,404.93 MT from a total harvested area of 36,670.21 acres with an average yield of 1.73 t/acre (RSD, 2019).

However, the production from within the country can meet only about 47% of the annual demand and the shortfall of more than 50% is met by importing rice mostly from India (Dema, Tashi, Ghimiray, & Chhogyel, 2019). Bhutan imported 61,467.8 MT of rice in 2018 costing Nu.1884.1 M (RSD, 2019). Rice sector is hit by challenges like drying out of irrigation water, unpredictable precipitation and, potential rice fields being fallowed – all attributed to climate change and global warming (Chhogyel & Bajgai, 2015). Ghimiray (2012) states that limited wetland, use of low yielding traditional varieties, inadequate inorganic fertilizer inputs, insufficient irrigation water and labour shortage are the main factors leading to the low productivity of rice in Bhutan.

Despite numerous constraints, rice is grown in all 20 dzongkhags at elevations ranging from 150 m in the southern lowlands to 2600 m in the north (Chhogyel & Bajgai, 2015). Rice growing agro-ecological zone of the country is categorized into four zones according to elevation and rainfall pattern namely, Wet subtropical (<600 masl), Humid subtropical (600-1200 masl), Dry subtropical (1200-1800 masl) and Warm Temperate (1800-2600 masl) (Ghimiray, Pandhey, & Velasco, 2013).

The warm temperate or the high-altitude rice production agro-ecology in Bhutan is very unique in that during the rice crop season this environment has a low-high-low temperature pattern similar to that found in Japan, Northern China and Korea (Ghimiray et al., 2008). Considering the very short growing season and a low-high-low temperature pattern, the critical selection parameters established for evaluation and selection of high altitude rice varieties include cold tolerance at the seedling stage, high spikelet fertility, medium maturity (140-160 days), preferred grain quality by farmers, high yield, and tolerance to pest and diseases - specifically rice blast (Ghimiray et al., 2008). The climatic conditions permit only a single crop of rice cultivation. Low temperature is a major constrain at nursery, reproduction and ripening stages. This production zone constitutes roughly 20% of the total rice-growing area in Bhutan, thereby substantially contributing to the country's rice production (Ghimiray et al., 2013). The warm temperate rice production zone is also known for high productivity as compared to other rice production agro-ecology in the country.

Bhutan has a history of an unprecedented blast epidemic that occurred in 1995 caused by the fungus *Pyricularia grisea*. The outbreak was most severe in high altitude regions of Thimphu and Paro though less severe incidences were also reported in mid-altitude areas (Thinlay, Finckh, Bordeos, & Zeigler, 2000). It was reported that most of the high elevation cultivars were found to be blast susceptible which led to the epidemic (Thinlay et al., 2000). Moreover, both Paro and

Thimphu districts experienced unusual weather patterns in 1995 which favoured the outbreak Thinlay et al., 2000). Following the blast outbreak, the government took measures to replace traditional rice varieties with varieties that possess high field tolerance to the rice blast disease, were high yielding with suitable maturity period fitting into the very narrow and specific high-altitude rice-growing environment, and with characters that were ultimately acceptable to the farmers.

With global climatic patterns becoming inconsistent, epidemics more virulent than the blast outbreak of 1995 could put the already threatened rice cultivation at risk in the future. This study was therefore aimed at establishing a minimum baseline for agronomic parameters of high-altitude rice varieties in relation to temperature and rainfall by relating the mean optimum required for rice as against the mean for the warm temperate rice production agro-ecology. The baseline parameters could then serve as a basis for future germplasm introduction, breeding and selection for warm temperate or high-altitude rice agro-ecology in Bhutan.

Eight popular high altitude rice varieties were selected for the trial to examine and establish the agronomic parameters. Ten years' weather data (2009-2018) of Paro Dzongkhag were collected from the National Centre for Hydrology and Meteorology (NCHM) to determine the weather parameters at different growth stages of rice.

2. Materials and Methods

2.1. Experimental site

The study was conducted during the 2019 rice season at Tsento-Shari rice research station in Paro Dzongkhag. The research station was leased in from the Farm Machinery Cooperation Limited (FMCL) in 2017 and is being managed by the Agriculture Research and Development Centre, Yusipang since then. The site is located at an altitude of 2450 masl which falls under “Warm Temperate” agroecological zone. The experimental site is very convenient in carrying out high rice research as most of the popular rice varieties under high altitude region thrive well at the site, and the site is also equipped with adequate field facilities.

2.2. Weather data

Ten years' weather data (2009-2018) of Paro district were collected from the National Centre for Hydrology and Meteorology (NCHM) website, and temperature and rainfall at different growing stages of high-altitude rice were studied. The temperature at high altitude rice-growing zone was also compared with the standard temperature required by rice for its optimum growth and development and the reasons for the differences in temperature between the two were discussed.

Table 1. Ten years weather data of Paro district (2009-2018); Source: NCHM (2018).

Month	Maximum Temperature (°C)	Minimum Temperature (°C)	Mean monthly Temperature (°C)	Maximum Rainfall (mm)	Minimum Rainfall (mm)	Mean Monthly rainfall (mm)
January	13	-3	5	5	2022	1014
February	16	-1	7	12	1768	890
March	18	3	10	20	1890	955
April	21	6	14	31	1789	910
May	22	10	16	64	1873	969
June	24	13	19	62	1911	987
July	24	14	19	101	2134	1117
August	24	14	19	94	2133	1114
September	23	13	18	72	2064	1068
October	21	8	14	40	1989	1014
November	17	3	10	1	1857	929
December	15	-2	7	2	2006	1004

2.3. Design and Layout

The trial was laid out in Randomized Complete Block Design (RCBD), and was replicated three times where the treatments were assigned randomly. There were eight treatments laid out in plots of 5 m x 2 m or 10 m^2. The seedlings were transplanted at plant to plant and row to row spacing of 20 cm x 20 cm.

2.4. Treatments

The treatments were: Yusiray Maap-1, Yusiray Maap-2, Yusiray Kaap-2, Yusiray kaap-3, Yusiray Kathramathra, Khangma Maap, Jakar Ray Naab and Janam, a traditional variety as a standard check. The treatments were chosen based on their popularity in high altitude region especially in Paro and Thimphu Dzongkhags.

2.5. Data collection

The trial was monitored regularly and data were collected at different stages of the crop. The traits on which data were collected include plant height, days to 50% flowering, number of tillers per hill, days to maturity and grain yield per plot. The trial was harvested from an area of 5.04 cm^2 after removing the borders. The grain moisture content at harvest ranged between 20-25%. However, plot yield was measured when the grain moisture was reduced to 15% or below.

2.6. Data analysis

The data was analyzed using Statistical Tool for Agriculture Research (STAR) software. Analysis of variance (ANOVA) was done to verify the variation in the traits.

3. Results and Discussion

3.1. Agronomic traits

Data on different agronomic traits were collected and comparisons of different traits were made to provide a general agronomic description of high-altitude rice. The standard check used was a popular a traditional variety Janam with a long history of adaptation to the warm temperate agro-ecozone. The comparison of the agronomic traits of improved varieties with that of the local variety provides strong evidence that the traits are suitable and acceptable to farmers of high-altitude rice-growing areas with very specific climatic requirements.

Table 2. Different agronomic traits of high-altitude rice varieties.

Variety	Plant Height (cm)	Days to 50% flowering	Number of tillers	Days to Maturity	Yield (t/acre)
Jakar Ray Naab	104^{c}	162^{e}	14^{a}	206^{f}	2.01^{b}
Janam	140^{a}	165^{d}	14^{ab}	210^{e}	2.26^{ab}
KhangmaMaap	119^{b}	168^{c}	12^{ab}	215^{d}	2.60^{ab}
Yusiray Kaap-2	120^{b}	170^{c}	11^{ab}	210^{e}	2.48^{ab}
Yusiray Kaap-3	104^{c}	180^{a}	10^{ab}	236^{a}	2.56^{ab}
Yusiray Kathramathra	107^{c}	181^{a}	13^{ab}	235^{a}	2.98^{a}
Yusiray Maap-1	120^{b}	177^{b}	13^{ab}	225^{b}	2.91^{ab}
Yusiray Maap-2	111^{bc}	170^{c}	10^{b}	221^{c}	2.61^{ab}
P-value	0.00	0.05	0.023	0.00	0.04
SE±	3.19	0.55	1.26	0.88	0.26
CV(%)	3.38	0.39	12.84	0.49	12.57

Values in columns followed by different letters in subscript are significantly different at 0.05 % level of significance.

Grain yield

The result (Table 2) show that Yusiray Kathramathra had the highest grain yield (2.98 t/acre) while Jakar Ray Naab had the lowest (2.01 t/acre). Due to slender culms, heavy lodging was observed in Jakar Ray Naab as a result of which the yield was affected.

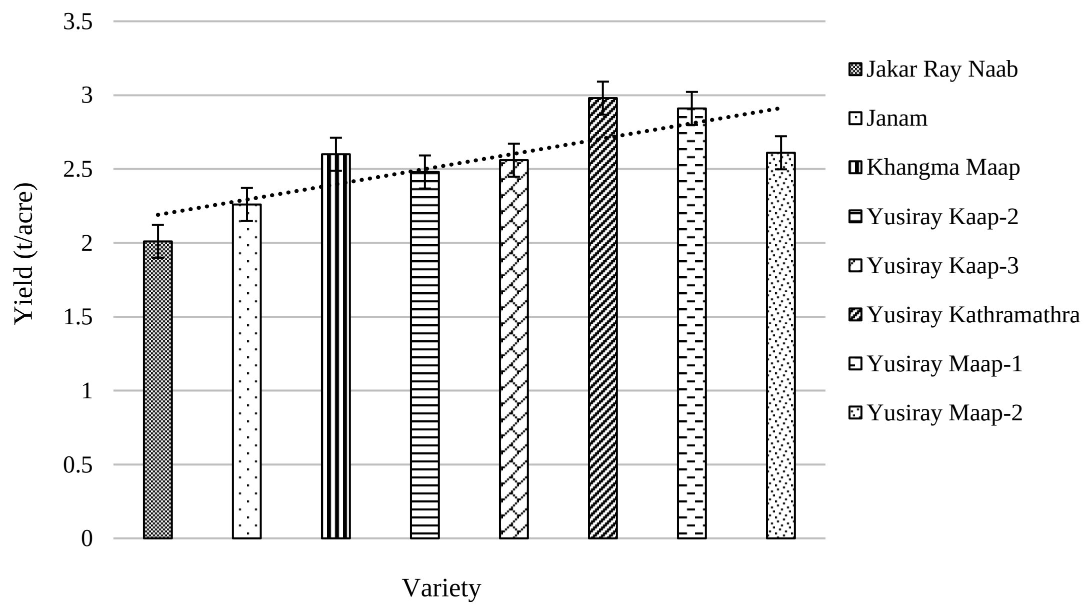

Figure 1. Yield of different high altitude rice varieties.

The analysis, however, showed that except between Yusiray Kathramathra and Jakar Ray Naab, no significant difference in yield was observed among other varieties. Though Yusiray Kathramathra had the highest yield, due to its smaller grain size, fewer farmers opted to grow it in their field while Jakar Ray Naab was found to perform better than other varieties at higher elevations (> 2500 masl).

Plant height

The study affirmed that plant heights for high altitude rice regime ranged from 104 cm to 140 cm with Janam being the tallest while Jakar Ray Naab and Yusiray Kaap-3 were the shortest. The experiment showed that Janam was significantly taller than the rest of the varieties depicting that tall stature is a common trait in traditional varieties.

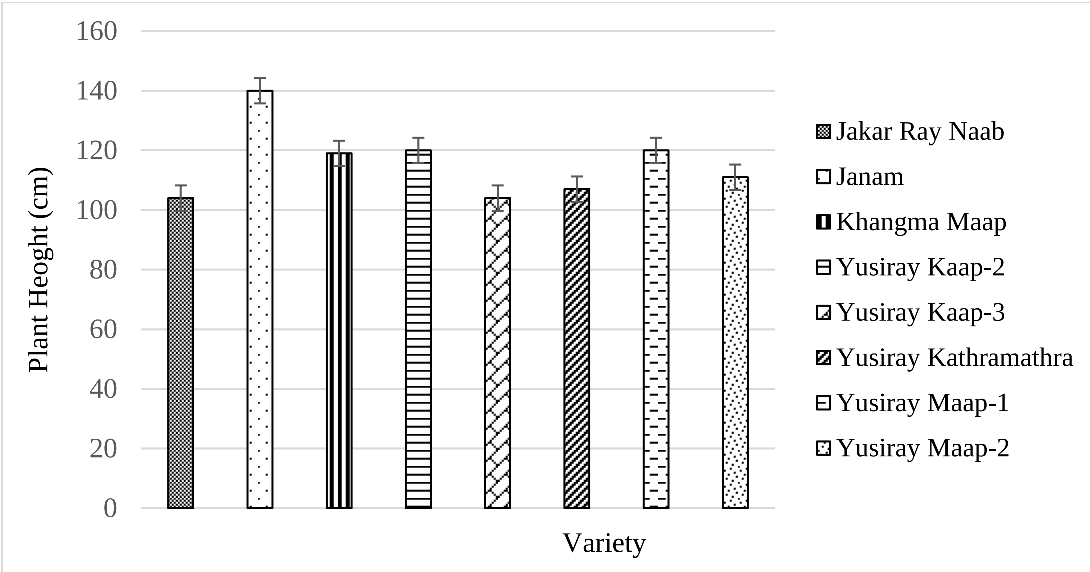

Figure 2. Plant height of different high-altitude rice.

Tillering

Since numbers of tillers per plant are strongly correlated to the number of panicles per plant, high tillering capacity is regarded as an important trait in rice production (Miller, Hill, & Roberts, 1991). Tillering in rice starts 10 days after transplanting and it attains maximum tillering stage 50-60 days after transplanting (Vergara, 1992). Chaudhary and Ghildyal (1970) reported that tillering is positively correlated with rising temperatures between the range of 15-33 ºC, but above 33 ºC, tillering is rendered unfavourable. According to Mahbubal Alam, Islam, and Muhsi (1985), the optimum temperature for tillering is 25-31 °C. The temperature during the tillering stage was observed between 12-24 ºC in high altitude rice-growing region. Though the temperature was found lower than the optimum temperature required, Yoshida (1981) revealed that rice plants have lower threshold temperature (10-13 ºC) during vegetative stage which makes them less sensitive to cold. Satake and Hayase (1970) indicate that decreased tillering, lower stature and yellowing of leaves are the common symptoms of chilling stress observed at the vegetative stage of rice. The experiment (Table 2) shows that Janam and Jakar Ray Naab had the highest numbers of tillers (14) while Yusiray Maap-2 had the lowest (10). The maximum tillering stage was observed in mid to third week of July depending on the variety.

Budhar and Palaniappan (1996) observed that the application of nitrogen fertilizer increased the number of productive tillers. Yoshida (1981) also mentioned that tillering is dependent on the nitrogen, phosphorus and potassium content in the leaf blades. Wang et al. (2017) found that late-emerging tillers had a lower number of spikelets per panicle and grain filling percentage and hence yield contribution was lower than early emerging tillers.

Maturity

The number of days to maturity was calculated from the date of establishment of nursery to the date of crop harvest. In the warm temperate agro-ecozone farmers establish the nursery by the second fortnight of February when the mean monthly temperature is around 7 °C which then picks up to only 0 °C in March (Table 1). The optimum temperature for germination of rice seed is 25-35 °C. Normally farmers in the warm temperate agro-ecozone transplant about 90 days old seedlings which allows the seedlings to attain the hardiness required to cope with low temperatures. It is also perceived that the rice plants gain some level of physiological maturity in the nursery that helps them tolerate the low mean temperatures at ripening. The days to maturity for the eight varieties ranged from 206 to 236 days from seed to seed. Jakar Ray Naab was observed to be the earliest variety to mature while Yusiray Kaap-3 took the longest duration to mature (Table 2). All the high-altitude varieties can be categorized as long duration varieties.

The optimum temperature for rice at ripening stage is 20-25 ºC with a critical low range of 12-18 ºC (Table 3). High-altitude rice matures in the month of October and harvest has to be completed by last week of October before the onset of early frost. The temperature in October in Paro which represents the warm temperate rice production zone ranged from 8-21 ºC with a mean value of 14 ºC (Table 1) which was within the lowest critical range. Any varieties with longer maturity than the tested varieties will show high spikelet sterility, empty grains and the panicles will not droop due to cold temperature.

3.2. Comparative analysis on temperature at different growth stages/phases of rice

The standard temperature (Table 3) required by rice for its optimum growth and development was compared with the existing temperature at high-altitude rice growing agro-ecology (Table 4) at different growth stages, and the possible causes for the difference in temperature between the two were discussed.

Table 3. Response of the rice plant to varying temperatures at different growth stages. Source: (Yoshida, 1981).

Growth Stage	**Critical temperature (ºC)**		
	Low	**High**	**Optimum**
Germination	10	45	20-35
Seedling emergence and establishment	12-13	35	23-30
Tillering	9-16	33	25-31
Anthesis	22	35	30-33
Ripening	12-18	30	20-25

Table 4. Temperature at different growth stages of high-altitude rice growing agro-ecology.

Growth Stage	Critical temperature (ºC)		
	Low	High	Mean
Germination	5	20	12.5
Seedling emergence and establishment	8	22	15
Tillering	14	24	18.5
Anthesis	13.5	23.5	18.5
Ripening	11	22	16.5

Vegetative Phase

a) Germination and seedling establishment

Farmers generally practice dry bed method of nursery in high altitude region. Rice nursery development begins with the onset of the warm season, i.e., by mid of February to mid-March. Seedling germination takes 3-4 weeks, prolonging the nursery period over 90 days. Dubey, Verma, Goswami, and Devedee (2018) reported that low temperature delays the germination of rice up to one month or longer because plants tend to reduce their physiological response during severe cold stress. Germination of rice seedlings can occur at a temperature range between 10-45 ºC with an optimum temperature range of 20-35 ºC and temperatures below 10 ºC could result in complete failure of germination (Yoshida, 1981). The mean temperature observed (Table 4) in high altitude rice-growing region during the germination stage was 12.5 ºC which is only slightly above the critical minimum temperature. Lou et al. (2007) stated that temperature below 15 ºC makes rice prone to damage by chilling stress, especially during the early seedling growth phase. Yoshida, (1981) reported that stunting is a common symptom of cold injury in rice seedling.

However, to protect the membranes from freezing damage, sucrose and other simple sugars are found to accumulate to acquire cold tolerance in most species (Thomashow, 1999). Cold tolerant rice cultivars were revealed to possess higher content of antioxidant and maintain higher activity of defensive enzymes (Huang & Guo, 2005). It was also indicated that higher gibberellic acid (GA) content in rice seedlings increased cold tolerance in rice cultivars (Naidu, Fukai, & Gunawardena, 2005).

b) Shoot elongation

Nishiyama (1977) stated that the critical minimum temperature for shoot elongation ranged between 7-16 ºC. By the third week of May when rice seedlings attain 2-3 leaf stage at 15-25 cm height, it is ready for transplantation in high altitude rice-growing environment. The temperature during transplantation increases to 17 ºC. A moderate increase in temperature was found to accelerate the emergence of leaf in rice (Gao, Jin, Huang, & Zhang, 1992). There is also adequate rainfall to augment consistent irrigation water supply during transplantation. The monthly rainfall was recorded between 63-1892 mm in May-June month. Sridevi and Chellamuthu (2015) reported that crop stand and the duration of crop growth is affected by variation in rainfall and the height of rice crop was taller when there was high rainfall during active growth period.

3.3. Reproductive phase (flowering)

The opening and closing of spikelet (floret) are referred to as anthesis and it lasts for about 1-2.5 hours. Anthesis normally occurs between 8:00 AM to 1:00 PM. Flowering in rice plant can occur between a temperature range of 22-35 ºC with the optimum temperature ranging between 30-33 ºC (Yoshida, 1981). In high altitude rice-growing region, the temperature during flowering time ranged between 13.5-23.5 ºC with a mean temperature of 18.5 ºC. The flowering stage of rice is most sensitive to high temperature (Tian, Tsutomu, Li, & Lin, 2007) followed by low temperature after booting. Temperature exceeding 35 ºC for more than 1 hour caused heavy spikelet sterility (Yoshida, 1981) while low-temperature range between 10-15 ºC was critical for spikelet fertility (Tinarelli, 1989). High temperature leads to incomplete splitting of anther, wilting of stigma and pollen desiccation causing fertilization failure (Osada et al., 1973) while low temperature interferes with the pollen grain formation (Tinarelli, 1989). The experiment showed that Jakar Ray Naab was the first to attain 50% flowering in 162 days while Yusiray Kathramathra was the last to attain 50% flowering in 181 days. The rainfall during flowering time was found to range between 83-2099 mm. Vijayakumar (1998) mentioned that continuous rainfall for three days would impact proper anthesis in rice. Heavy rainfall was found to result in a large number of empty spikelets (Sreenivasan, 1985).

3.4. Ripening Phase

The experiment showed that Jakar Ray Naab was the earliest to mature (206 days) while Yusiray Kaap-3 (236 days) took the longest duration. The temperature requirement for ripening phase ranges from 12-30 ºC with an optimum range between 20-25 ºC (Yoshida, 1981). The temperature at ripening/maturity stage in high altitude rice-growing zone ranged from 11-22 ºC with a mean temperature of 16.5 ºC. (Tashiro & Wardlaw, 1989) found that lower temperature during maturity/ripening stage resulted in the heavy shattering of grains and also lengthened the ripening period as translocation of photosynthates to the grains are slowed down. When the temperature drops below 10 ºC for several days, the ripening process is found to stop and due to cold stress maturity is largely delayed (UAS, 2018). On the other hand, high temperature during grain filling period triggers photorespiration (Khan, Kumar, Hussain, & Kalra, 1999) and high temperature is also found to cause decreased grain weight due to the inability of the spikelets to serve as a sink during temperature stress (Oh-e, Saitoh, & Kuroda, 2004).

4. Conclusion

Constituting approximately 20% of the total rice-growing region in the country, the warm temperate high-altitude agroecology has a very essential role in achieving the nation’s food security. Moreover, our rice farmers face challenges like drying up of irrigation water sources, conversion of wetland to dryland due to developmental activities, paddy fields getting fallowed over time, multifold increase in wages and the younger generation not taking up farming. Moreover, with climate and weather patterns becoming more unpredictable, a need to evaluate and establish the agronomic parameters of rice varieties for the warm temperate rice–ecozone in relation to temperature and water availability at different growth stages has become very critical.

This study on agronomic parameters of high-altitude rice varieties gives a very broad indication that temperature range during the growing season falls in the lowest critical range and rice varieties require very specific adaptation features. The 10-year weather data used in this experiment clearly indicate that the temperature range in the warm temperate rice growing environment is much lower compared to the optimum temperature required by rice plant for optimum growth and development. Lower soil, air and irrigation water temperatures lead to a long and extended maturity period in high altitudes. Nursery period in the warm temperate region was found to be very lengthy (about 90 days) due to prevailing low temperature during those months (February to April). Rapid seedling growth was observed only by April when maximum temperature was recorded at 20ºC. The temperature at flowering stage was also found to play a very critical role as very low temperature interferes with pollen grain formation. Continuous rainfall during flowering also leads to a large number of empty spikelets, ultimately hampering production. Due to the cold low-high-low temperature pattern in warm temperate agro-ecozone, all rice varieties have to possess specific agronomic parameters, mainly cold tolerance at the seedling stage, medium maturity duration, high spikelet formation, preferred grain quality by farmers, and high yield and tolerance to pest and diseases. Future studies have to take into consideration long term climate data and its correlation to specific agronomic parameters of high-altitude rice varieties that help them perform under such a production environment. This will help rice breeders to develop more climate-resilient varieties suitable for the warm temperate agro-ecozone.

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Effect of Fruit Thinning on 'Hosui' Pear Fruit Quality and Yield

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ABSTRACT

In Bhutan, naturally occurring local type of pears were grown. Asian or Nashi pears are recent introduction which have become quite popular in eastern Bhutan since it was promoted by Agriculture Research and Development Centre (ARDC) Wengkhar. Although, horticulture has been gaining momentum in recent years, many growers in Bhutan follow traditional methods of crop cultivation and thus, majority of farmers still do not adopt important aspects of fruit production like fruit thinning. In commercial fruit farms elsewhere, fruit thinning is an essential management practice. However, in Bhutan, it is rarely practiced and there is no empirical study on its benefits. Fruit thinning is believed to be beneficial in increasing yield efficiency whilst also sustaining or increasing market returns by improving the fruit size and quality. Thus, a study was conducted in 2019 and 2020 at ARDC, Wengkhar. Effect of hand thinning on Hosui pear was assessed by randomly sampling 15-year-old Hosui trees with four replications – each tree treated as an experimental unit. Canopy of these trees were divided into two equal halves so that one half represents thinning and the other half non-thinning treatments. The treatments were randomly assigned to the two halves of each tree. Hand thinning resulted in significant increase in the fruit weight by 39%, fruit diameter by 12% and fruit height by 12 % over the non-thinning treatments; Total Soluble Solids (TSS) content, an indicator of taste, was greater by 9%. However, the overall yield was lower in the thinning treatments but in the second year of the study, the mean fruit yield in thinning treatments increased by 55% while the yield of non-thinning treatments plummeted by 33%. Hence, the study indicates that fruit thinning has significant effect on fruit quality and yield stability.

Keywords: *Fruit firmness; Fruit size; Fruit yield; Hand thinning; Hosui; TSS*

1. Introduction

In general, pears are grown between an altitude of 955 to 2700 m above mean sea level in the country but Asian pear gained popularity in the east after its introduction to ARDC Wengkhar in 2002 (Phuntsho et al., 2011). In Bhutan, it was mostly the naturally occurring local type with egg sized fruit with hard pulp (Brix of 12 %) consumed after drying (Phuntsho et al., 2010). A robust promotional program on horticulture in the eastern region led to farmers taking up mixed fruit farming of pear, peach, plum and persimmon (Katwal, 2013) but agriculture in Bhutan is still traditional in nature and majority of farmers are illiterate so research and extension programs play key role in the promotion of vibrant agricultural sector (Tobgay, 2006).

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Fruit thinning is one of the critical aspects of commercial fruit farming in many developing and advanced countries. However, in Bhutan, it is rarely practiced and farmers are yet to realize its real benefits. Fruit thinning basically means removal of excess fruits especially those that are misshaped, weak and diseased so as to maintain ideal ratio of leaves to fruits numbers so that fruits are supplied with adequate amount of metabolites. In general, almost all fruits exhibit on and off years resulting in heavy production one year and very low production in the following year. Thus, pear orchards often have problems of vigorous growth and production in one year followed by biennial bearing in the ensuing year (Lafer, 2007). Biennial bearing behavior of fruit trees is also reported by other studies and it is considered as undesirable (Jonkers, 1979). Thinning of fruits during the "on" year has been found to be valuable means to overcome alternate bearing in fruit trees (Monselise & Goldschmidt, 1982). Hence, fruit thinning remains one of the most important and effective cultural management tools to not only avoid undesirable effects of biennial bearing but also to improve fruit size and fruit quality (Greene & Costa, 2012).

Fruit thinning can be done both through use of chemicals and manually. Hand thinning is considered the most reliable although it is the most expensive method (Costa, Blanke, & Widmer, 2012). According to a study by Duhanaj, Susaj, Roshanji, and Susaj (2015), even taking into consideration the cost of hand thinning, it can be recommended especially for small growers and small areas under cultivation like in Bhutan. For chemical thinning, the main drawback is the unpredictability of the results arising from weather and tree factors which might hinder its efficacy (Wertheim, 2000). Hand thinning is an environmentally acceptable method of reducing crop load (Chabikwa, 2008) and it supports the country's efforts towards going organic (Duba, Ghimiray, & Gurung, 2008). Even though, chemical thinning might seem alluring due to its labor efficiency and reduced cost, hand thinning is the way forward for Bhutanese farmers in view of the country's orientation towards organic farming.

2. Materials and Method

The trial was established at a research orchard block at ARDC Wengkhar located 1583 m above mean sea level (27°16. 315' N,091°16.214'E), Mongar, Bhutan. The experiment was carried out using completely randomized design (CRD) with one factor having two levels - thinning and non-thinning treatments. The study used four 15-year-old Hosui trees. Each tree was divided into two parts. Thinning and non-thinning treatments were allotted randomly to each part of the tree which amounted to four thinning treatments and four non-thinning treatments. Fruit thinning was performed with the help of a Japanese Okatsune thinning/harvest shear.

Fruit thinning is usually carried out after the June natural drop. However, physiological fruit drop leads to a reduction of the fruit number per tree (Costa et al., 2012) and early thinning reduces natural drop of remaining fruitlets; so the severe thinning before the first drop reduces the need for later hand thinning (Burge, Spence, & Dobson, 1991). Further, early thinning treatments tend to increase fruit size by causing some stimulation in cell volume in addition to a marked increase in cell number (Sharples, 1968). Hence, the first and second fruit thinning were carried out by the 6th

and 29th of May respectively in both the years. Hand thinning helps eliminate fruits with poor characteristics, such as undersized, dark-green fruit close to the tree trunk or scab or hail affected fruits (Costa et al., 2012). Trees should be thinned so that the potential difference in size can be manifested at harvest (Burge et al., 1991), and therefore, undesirable fruits were removed to maintain only 2 to 3 fruits per each fruit cluster on the experimental units for thinning treatment. The experimental units were covered with a bird net to manage bird damage to fruits.

Fruits were harvested on 12th August in 2019 as well as in 2020. The yield of each experimental unit was measured, and 20 fruits from the eight experimental units were collected for analysis of quality parameters like fruit weight, fruit height, fruit diameter, TSS and firmness. The fruit weight was measured with a digital weighing balance. Fruit height and fruit diameter were measured with a digital vernier caliper. TSS was measured with digital refractometer while a penetrometer was used to measure fruit firmness.

Data was recorded in Microsoft Excel sheet and the Statistical Tool for Agricultural Research (STAR) software was used to analyze pooled data for two years using Analysis of Variance (ANOVA) model.

3. Results and Discussion

Effect of thinning on fruit quality and yield are discussed in terms of fruit quality parameters like TSS, weight, height, firmness and stability of yield.

3.1. Fruit Weight

Reducing levels of fruit set is desirable in order to reduce inter-fruitlet competition for the tree's available resources. Excessive fruit set often results in poor fruit size at harvest (Webster, 2000) but hand thinning induces the production of heavier or larger size fruits (Stampar & Hudina, 2007). Studies conducted have negatively correlated mean fruit weight with crop load (Link, 2000) and the mean fruit weight was found to be increased by hand-thinning treatment (Bound, 2000). Bigger fruits exceeding 170 g fetch better price in the market (Buwalda, Klinac, & Meekings, 1989). In our study, the average weight of fruit from thinning was found to be 273.8 g while the mean weight for fruits from non-thinning treatment was 197.4 g. ANOVA analysis found that thinning produces significantly heavier fruits by LSD ($P < 0.001$) (Table 1). Large pear fruit size is critical to economic success in many markets (Dussi & Sugar, 2010) which can be achieved by thinning of fruits. Fruit thinning consequently helps in fetching good economic return.

3.2. Fruit size (Height and diameter)

Fruit size is also one of the important criteria. Consumers often prefer bigger fruits over smaller ones. Where fruit set is excessive and natural abscission of fruitlets inadequate, methods of increasing their abscission must be employed if large fruit size at harvest is to be achieved (Webster, 2000). The practice of hand thinning can be beneficial to increase fruit size and color by singling fruit within the cluster; it also reduces the incidence of pest and diseases (Robinson, Lakso, Greene, & Hoying, 2013). As indicated in Table 1, fruits from thinned treatments are significantly greater in both mean height and diameter (P<0.001). The mean height of fruits on the thinned treatments was 6.9 cm and for the non-thinning treatments was 6.2 cm. The mean diameter of fruits from experimental units subjected to thinning was 8.2 cm in comparison to 7.3 cm for those from the non-thinning experimental units. This clearly indicates that fruit thinning at appropriate time can significantly increase the fruit sizes.

3.3. Fruit TSS

One of the most important parameters in fruits is TSS, which indicates the taste of the fruits. Usually, higher the TSS, better the quality is. In our case, on average, fruits from thinning treatments had 12.1%-degree brix while, non-thinning treatments had 11.1%-degree brix (Table 1). As per our study, fruits from the thinning treatments achieved significantly higher TSS as compared to the non-thinning treatments (P<0.001). This result is in conformity with the study that found hand thinning had an influence on soluble solids, increasing them in both the years of study (Stampar & Hudina, 2007). In another study, it was seen that the sugar content of the fruit increased significantly with fruit thinning (Maas & Van der Steeg, 2010). Similarly, fruit thinning increased the TTS in fruits which in turn improved the taste (Link, 2000). Therefore, all these studies clearly show that fruit thinning helps in improving TSS in addition to fruit weight and size.

3.4. Yield

Consistency in yield is an important factor in commercial farming. For the investment in pear orchard to be justified, it is essential to increase yield efficiency, whilst also sustaining or increasing market returns by improving the fruit size and quality grade outs (Webster, 2000). In our study, thinned treatments had an average yield of 28.1 kg while non-thinned treatments had 51.7 kg per tree (Table 1). The average yield from the thinned treatments is significantly lower in comparison with non-thinned treatments (P=0.016). This is also in agreement with what other studies have reported. One of the studies reported that yield per tree and per hectare was significantly lower following hand thinning (Stampar & Hudina, 2007).

However, this low yield can be compensated by high quality fruits which fetch better prices than by non-thinned fruits. Further, although thinning treatments gave lower yield as compared to the non-thinning treatments, in the second year of our study, thinned units gave significantly higher average yield of 34 kg per unit compared to 22.3 kg in the previous year. In the case of the non-thinned units, in the second year, the average yield per unit dropped drastically from 61 kg per tree to 42 kg per tree (Figure 1). This clearly indicates that in the long run, thinning trees will give not only better fruits in terms of quality and size but also yield stable production over the years unlike non-thinned trees that yield unstable production with poor quality fruits. This result is also in consonant with what other studies have reported. Thinning results in fewer kilos of small and more kilos of large fruits (Link, 2000), and the increasing economic value per unit fruit weight with increasing fruit size demonstrates the merit of carrying out thinning (Buwalda et al., 1989). Therefore, the fruit yield might be reduced after fruit thinning but it helps to produce more marketable fruits. To keep producing marketable fruit and to ensure regular cropping it is essential to reduce the number of developing fruits in "on" years (Menzies, 1980).

Buwalda et al. (1989) reported that leaving trees un-thinned increased the incidence of natural drop before harvest, and this presumably resulted in the reduced fruit yield, which may be responsible for the apparent yield drop in non-thinning treatments. For the thinning treatments, as stated by Webster (2000), reducing level of fruit set due to thinning might have reduced fruitlet competition for the tree's available resources which led to the increased production.

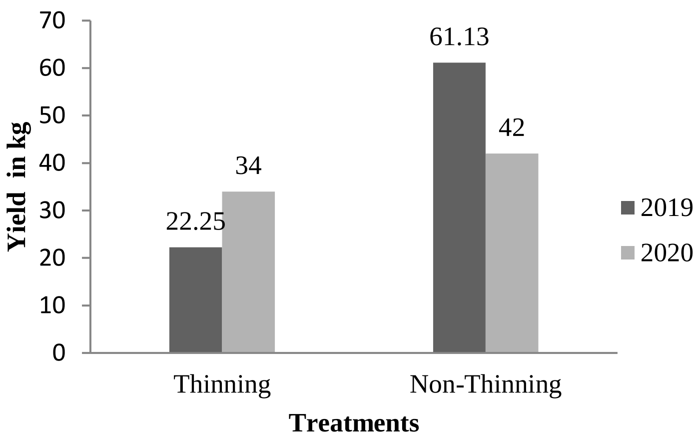

Figure 1. Yield variation in thinning and non-thinning treatments in 2019 and 2020

3.5. Fruit firmness

In the case of fruit firmness, there was no significant difference between the two treatments (*P* =0.075) though non-thinning treatment had slightly higher firmness than thinned ones.

Table 1. Comparison of means of fruit quality parameters for thinning and non-thinning treatments.

Treatments	Fruit weight(g)	Height(cm)	Diameter(cm)	TSS (%)	Firmness(kg/cm^2)	Yield(kg)
Non-thinning	197.4^b	6.2^b	7.3^b	11.1^b	0.67	51.6^a
Thinning	273.8^a	6.9^a	8.2^a	12.1^a	0.57	28.1^b
P value	<0.001	<0.001	<0.001	<0.001	0.075	0.016

Superscripts with dissimilar alphabetical letters are significantly different at alpha level 0.05.

Overall, thinned trees produce more marketable fruits which fetch better prices in the market. Thus, superior quality fruits from the trees which had undergone fruit thinning fetch Nu.50 per kg in the local market whereas fruits from the trees which had not undergone fruit thinning fetch Nu.30 per kg. In due course of time, overall returns from thinned trees are likely to increase further since thinning helps in maintaining the health and productivity of the trees.

4. Conclusion

Hand thinning of Hosui pear had a positive impact on fruit quality parameters like fruit weight, fruit diameter, fruit height and TSS. The mean fruit weight for thinning treatments was 38.7% greater than that of non-thinning treatments. Average TSS of thinning fruits was 9% higher than the non-thinning fruits which contributed to sweeter taste in thinned treatments. The fruits from the thinned treatments were apparently bigger than the ones from the un-thinned treatments. As for the fruit yield, the thinned treatments had lower productivity in the first year of trial but there was a remarkable increase in the second year whereas, the fruit yield decreased for the no-thinned treatments in the second year of study. This might imply that, if the trees are left un-thinned, it will lead to reduction in yield over the years.

Thus, we can infer that hand thinning will aid in the production of bigger fruits with better taste. Hand thinning will also help mitigate the problem of alternate bearing in pome fruits like pear. Hand thinning might require more labor and time as compared to chemical thinning but chemical thinning is unreliable and there is health and environmental cost to it which is against the country's effort to go organic in the long run.

Hand thinning can be performed by pear farmers in Bhutan as the number of trees is not much as compared to huge commercial farms in other countries. Also, hand thinning is a precise and an organic way to remove the crop load in line with the organic vision of our country. Hand thinning can be performed without any adverse effect on the environment and it will lead to production of quality produce as borne out by this study. Hence, hand thinning in fruits need to be encouraged by all the fruit growers in the country.

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Evaluation of Sowing Time of Quinoa in a Potato-based Cropping System in Cool temperate Agro-ecological Condition of Haa, Bhutan

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ABSTRACT

Quinoa (Chenopodium quinoa) is a native crop of South America that has shown high adaptation potential under Bhutanese conditions since its first introduction in 2015. Globally, it is referred to as the healthiest food due to its high nutritional content. The study aimed to investigate best sowing dates for quinoa and determine the possibility of cultivating it as a second crop after potato in Haa. The research was conducted in Katsho Gewog (~2700 masl) in Haa district which falls under cool temperate agro-ecology in Bhutan. The experiment was laid out in a randomised block design to test two quinoa varieties: Amarilla Marangani (AM) and DOA-1-PMB-2015 (DOA). They were planted at five sowing dates from 15 June to 15 August 2019 at 15 days interval. Sowing dates had a positive effect on the growth and productivity of quinoa due to differences in temperature and precipitation. Highest seed yield of both the varieties was obtained when quinoa was sown on 15 July, suggesting best sowing date of quinoa between 1st week to mid-July. Amarilla variety provided the highest grain yield at 409.30 kg/acre with a corresponding highest plant height of 77.31 cm for 15th July sowing. Similarly, DOA's highest yield was 369.63 kg/acre with a plant height of 56.60 cm for the 15th July planting. The least yield and plant height were obtained for quinoa planted on 15th August. Hence, sowing Amarilla Marangani variety later than 15th July in Haa risks the crop to extreme winter temperatures coinciding with its critical growth stage. Quinoa as a second crop after potato could be possible provided sowing is timed before 15 July. Another alternative could be the use of relay cropping of quinoa with potato if due care could be taken during potato harvest which is a gap for further study. However, DOA variety could be used for late July sowing due to its shorter growing duration.

Keywords: *Quinoa; Sowing date; Bhutan; Agro-ecological zones; Yield of quinoa; Weed pressure*

1. Introduction

An ancient food of the Andean region of pre-Columbian population, quinoa (Chenopodium quinoa) is known for its huge genetic variability and adaptability in diverse agro-ecological conditions ranging from drier areas (350 mm precipitation) in higher altitudes (above 3500 masl) to colder temperatures (average temperature of 12 °C) (FAO, 2011). According to Rojas et al. (2015) there are 16,422 accessions of quinoa and its wild relatives (*C. quinoa, C. album, C.*

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berlandieri, C. hircinum, C. petiolare, C. murale and *Chenopodium* sp.) worldwide, which are conserved in 59 gene banks distributed in 30 countries. Gene banks in the Andean region conserve over 88% of the crop's accessions. Therefore, it is potentially a strategic crop that most studies account would play a vital role in food security and sovereignty. Quinoa is increasingly known for its highly nutritious food products. It has been in cultivation for several thousand years in South America with a high potential of adaptation success in its recent introduction in other countries (Hirich, Choukr-Allah, & Jacobsen, 2014). A pseudo-cereal and an annual broad-leaved plant characteristically 1–2 m tall with deep penetrating roots, quinoa is distinct from other cereals due to its outstanding protein content rich in amino acids, lysine and methionine. Quinoa grain is used in many different ways such as quinoa flour, soup, breakfast, cereal and alcohol, while the flour is utilized in making biscuits, bread and processed food. Its ability to produce high-protein grains under ecologically extreme conditions makes it important in diversification of future agricultural systems, especially in high-altitude areas of the Himalayas and north Indian plains (Bhargava, Shukla, & Ohri, 2006).

Bhutan first introduced quinoa in 2015 through the FAO's support with the prime objective of enhancing food and nutritional security through adaptation of this versatile crop in its mountain agriculture system and as a climate-resilient crop to diversify the existing traditional potato and maize-based cropping systems. With the first publication of quinoa research in an international journal, Bhutan successfully evaluated and released four quinoa varieties that adapted best in the country's mountain agro-ecology ranging from 640 masl to 2600 masl (Katwal & Bazile, 2020). Besides its exceptional nutritional qualities, quinoa exhibit tolerance to frost, salinity, and drought, and the ability to grow on marginal soils (Bhargava et al., 2006). With its marked adaptability to various growing conditions, the crop has the potential to fight hunger and malnutrition (FAO, 2011) - primary reasons for efforts into introducing the crop in Bhutan.

The success of quinoa cropping depends on selecting suitable sowing or planting time because the rate of emergence of seedlings has a direct bearing on the plant density and final yield. Seeds are therefore sown after understanding site-specific conditions including the location, varietal properties, soil moisture and sowing depth (FAO, 2011). Bhutan has a typical mountain agriculture farming system with numerous geographical challenges. These include typical small scale agricultural subsistence farming system with low levels of farm mechanization and abiotic stresses through intermittent forces of climate and other socio-economic constraints. The country is widely dominated by rugged and steep topography, giving rise to very large altitudinal variation ranging from 100 meters in the south to as high as 7,000 masl in the north. The country is divided into three distinct climatic zones: Alpine, Temperate and Subtropical (Katwal & Bazile, 2020; Neuhoff, Tashi, Rahmann, & Denich, 2014). Katwal and Bazile (2020) in their study at two locations representing two of these agro-ecological zones found that quinoa crop maturity and yield vary significantly both within and between varieties and suggested the need for a package of practices for the crop when grown under different agro-ecological conditions, including separate parameters for organic production and production in marginal environments.

The crop's resilient agronomic traits for drought and low soil fertility (Galwey, 1992) as well as its non-susceptibly to diseases of other cereals, and the crop being only slightly susceptible to soil-borne nematodes (S. E. Jacobsen, Mujica, & Jensen, 2003) may be of great value as a "break crop" in crop rotation between cereal crops and after potato. Katwal and Bazile (2020) in their recent findings on geographical suitability of quinoa in Bhutan indicate cool temperate region (2600-3000 masl), covering districts like Haa to be marginally suitable (20-40%) as compared with other climatic regions of warm temperate (1800-2600 masl), dry subtropical (1200-1800 masl), wet and humid subtropical (<1800) regions classified as suitable (40-60%), very suitable (60-80%) and excellent (>80%), respectively. In cool temperate region, frosts occur from the second fortnight of October until the first fortnight of April. The authors observed frost damage in quinoa when sown in the first fortnight of March and in August at Yusipang which falls in the cool temperate zone. Their preliminary observation on growing quinoa after potato in the warm and cool temperate areas indicated that sowing of quinoa in mid-July escaped frost damage at anthesis.

The present study investigates the best sowing date of quinoa in cool agro-ecological condition by selecting Haa district as a representative study site to determine the possibility of cultivation as a second crop after potato which is the main crop. Adaptation of quinoa after potato could also help diversify potato-based cropping system in the cool temperate agro-ecology. Other advantages include providing optional crop to generate a continuous source of income besides utilizing residual fertilizers in the soil following potato cropping.

Two varieties - Amarilla Marangani and DOA-1-PMB- 2015 - were selected for the study. The choice of the variety was based on results of the study conducted by Katwal and Bazile (2020) where the variety DoA-1-PMB-2015 was found to be the earliest, maturing within 100 days after sowing. Although Amarilla Marangani is observed to be late maturing, taking over 197 days, it was chosen for the trial based on its wide preference both by consumers and growers due to its bolder grain size. Besides, the inclusion of both early and late varieties could indicate optimal sowing time for the other varieties whose crop duration is between 100 and 197 days.

2. Materials and Methods

2.1. Experimental site and set up

The research was carried out as the part of the Food Security and Agriculture Productivity Project (FSAPP) on leased land from a farmer at Wangtsha village, Katsho gewog in Haa located at an elevation of over 2700 masl. Two varieties Amarilla Marangani (AM) and DOA-1-PMB- 2015 (DOA) were grown between 15 June and 15 August 2019. The choice of these varieties was based on the early maturing characteristics of DOA variety and the growers' preference for AM variety though it matures late. Experimental units of 15 m^2 area each were set up in a completely randomized block design (CRBD) with three replications for each sowing date. Ten rows were established in each plot, 30 cm apart, seeds sown at 2 kg of seeds per acre. All treatments were provided with the same land preparation as well as hand weeding operations during the growing period. Seeds were sown at 15 days interval from 15 June to 15 August, thereby comprising of five separate sowing dates in total. These were repeated three times in the experimental design. Temperature and rainfall data were requested from Weather and Climate Services Division, National Centre of Hydrology and Meteorology (NCHM), Thimphu since the research centre did not have temperature sensors to measure air temperature.

2.2. Data collection

Final plant heights were measured during trial harvest and grain yield was taken after harvested panicles from respective plots were cured and pre-dried for 15 days. A total of 10 plant samples were selected randomly from each plot for plant height. The first treatment from the 30th June sowing was wiped out by leaf miner infestation leaving no plants for measurement. The remaining treatments were provided with one spray of cypermethrin 50 EC at 15 days after sowing which helped save the remaining four treatments. Therefore, plant height and grain yield data are presented for only four planting dates.

2.3. Statistical Analysis

Statistical software IBM SPSS Version 22 was used to analyse the data for plant height and grain yield. The data were assessed for the fulfilment of assumptions of ANOVA (analysis of variance) before statistical analysis. ANOVA was used to detect the effect of treatments with statistical significance at P-values < 0.05 (alpha level). Means were separated using Tukey's Honestly Significant Different test.

3. Results and Discussion

3.1. Grain yield and days to maturity

The first treatment was entirely damaged by leaf miner despite spraying neem oil in an attempt to control the pest organically; suggesting neem oil was not effective in controlling leaf miner infestation in quinoa. Remaining treatments were saved by spraying synthetic insecticide. The analysis of the remaining treatments showed significant differences between sowing dates in terms of total grain yield and plant height (Table 1 and Figure 1). The highest plant height (77.31 cm) was obtained in quinoa sown on 15 July for Amarilla variety which also had the highest grain yield at 409.30 kg/acre. For DOA variety sown on the same day (15 July) the highest grain yield was 369.63 kg/acre with a mean plant height of 56.60 cm, which was slightly shorter than that of the highest plant height (65.13 cm) from the same variety planted on 30 July.

The longest growing season for quinoa was observed in plants from seeds sown on 15 July for both the varieties (144 days to full maturity) and the shortest from those sown on 15 August (84 days to full maturity). Days to maturity increased from 30 June to 15 July and decreased from 30 July to 30 August sowing dates for both the varieties. The grain yield and quality from treatment sown on 15th August was insignificant for both the varieties. By varietal nature, DOA is an early maturing than AM variety. However, the same days to maturity with AM variety (108 and 84 days) for 30 July and 15 August sowing, respectively was because DOA could not be harvested during its harvest schedule separately and had to wait for its harvest together with AM variety. Katwal and Bazile (2020) studied maturity duration for AM and DOA varieties in cool temperate agro-ecology and found that DOA variety took about 100days and AM 197 days to mature. While days to maturity of DOA variety somewhat conforms with the study, AM variety sown on 15 July was harvested 52 days earlier to the days to maturity of 197 days reported by Katwal and Bazile (2020).

To lend context and provide a comparative understanding of the grain yield from the study and actual production in farmers' field, the total area of quinoa under cultivation, production and productivity per acre in Bhutan is provided in Table 2. The yield from the study was significantly higher for both the varieties as compared to the national average. However, grain yield (409.30 kg/ac) and plant height (77.13 cm) obtained from plots sown on 15 July were considerably low as compared to grain yield of 582 kg acre^{-1} and plant height of 1.88 m for Amarilla Marangani variety and grain yield of 620 kg acre^{-1} and plant height of 120 cm for DOA-1-PMB-2015 variety reported by Katwal and Bazile (2020). Nevertheless, the low yield results from this study agree with the statistics provided by the Ministry of Agriculture and Forests (MoAF) (RSD, 2019) where the production in 2018 was merely 21 MT from 114.14 acres and just 9 MT from a cultivated area of 73 acres in 2017 (RSD, 2018) – indicative of poor yield. The low yield could be due to heavy weed pressure during the growing season, lack of farmyard manure or inorganic fertilizer, and the study site being largely stone-filled (about 40%). The yield was also adversely affected by aphids and leaf miner infestation which is the primary challenge in quinoa cropping in the cool temperate agro-ecological zone.

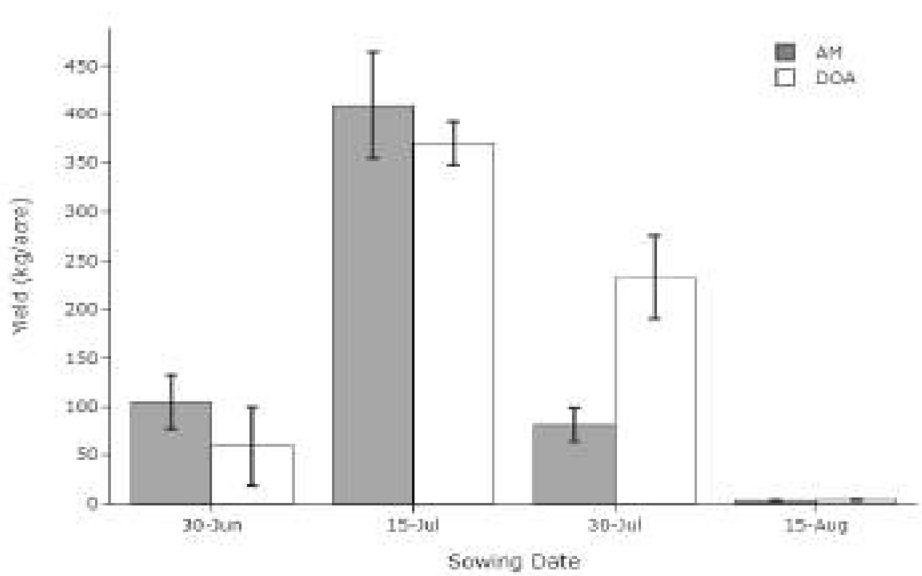

Figure 1. Yield of two quinoa varieties (AM and DoA) at different sowing dates. Error bars represent SE of means.

Table 1. Plant height (cm), grain yield (kg/acre), and days to maturity.

Sowing date	Variety	Plant height (cm)	Std. Deviation	Grain yield Kg Acre^{-1}	Std. Deviation	Days to maturity
30th June	AM	76.23^{a}	3.03	105.22 ^{b}	47.43	138
	DOA	34.93^{b}	5.2	59.36 ^{b}	70.56	111
15th July	AM	77.13^{a}	6.77	409.30 ^{a}	94.87	144
	DOA	56.60^{a}	6.84	369.63 ^{a}	38.06	123
30th July	AM	54.60^{a}	7.73	81.84 ^{b}	30.33	108
	DOA	65.13^{a}	14.01	232.93^{ab}	74.4	108
15th Aug	AM	26.20^{b}	3.51	3.62 ^{b}	0.72	84
	DOA	24.73^{b}	3.32	4.16 ^{b}	0.94	84

Values in the same column followed by different letters in subscript are statistically significant at $P<0.05$.

Table 2. Harvested area, production and yield of quinoa from 2014-2018 in Bhutan.

Year	Harvested Area (acres)	Production (MT)	Yield (kg/acre)
2017	73	9	123.288
2018	114	21	184.211

Source: (RSD, 2018)

3.2. Plant height

Plant heights were measured on 10 randomly selected plants from each plot. Height variation for each sowing date is presented in Figure 2. Significant difference in plant heights was observed amongst the sowing dates tested (as presented in Table 1 and Figure 2). The tallest plant height (77.13 cm) was observed in quinoa sown on 15 July for AM variety and 30 July for DOA variety while the same varieties sown on 15Augusthad significantly shorter plant heights. Correlation analysis (r =.690, $n=14$, $p=000$) indicate a strong and positive correlation between plant height and grain yield which agrees with the findings of (Hirich et al., 2014) who reported that plant height had a positive effect on total dry matter and grain yield. Studies have deduced grain yield of quinoa being directly proportional to its biomass.

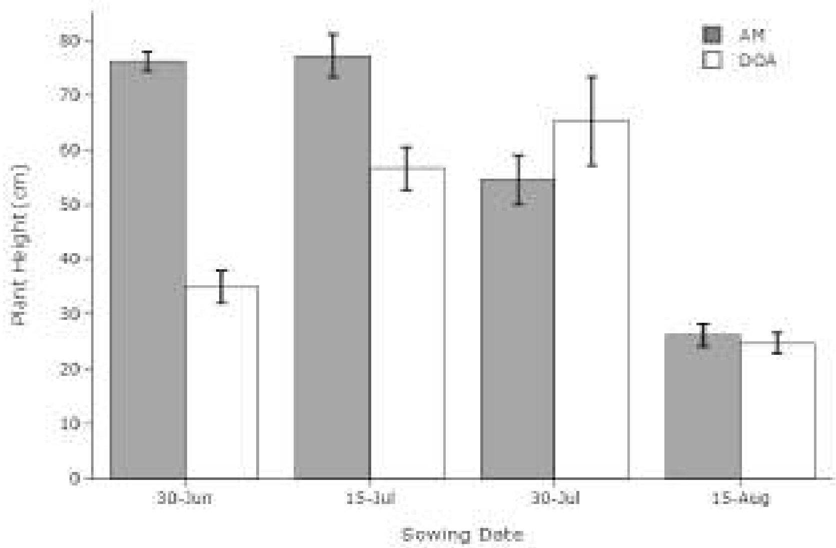

Figure 2. Variation in final plant height of the two quinoa varieties AM and DOA. Error bars represent SE of means.

3.3. Rainfall

Weekly rainfall variation during the growing period is presented in Figure 3. Very low and inadequate precipitation that was uniform in its range (between 11 – 14 mm) was recorded from June until the second week of August. Recorded rainfall is thus low with the onset of the dry season beginning 2nd week of August until December. This indicates that quinoa can be successfully promoted under cool temperate environment but with supplementary irrigation for improved and quality grain yield. Monthly rainfall data from Haa district between the years 2014 to 2018 is provided in Table 3 to relate the low precipitation as observed at the study site. Jacobsen (2003) tested the length of the growing period of quinoa in Peru and Denmark. It was observed that there was no significant difference among cultivators in the length of the growing period within a given year, but the growing period actually differed significantly between years. The study in 1991 showed that the crop growth was slow with a long growing period due to wet and cold spring and early summer, while drought spell in the following year in 1992 in the months of May–June favoured quick crop development.

The yield from the trial could have been adversely impacted by the dry season, and low and uneven rainfall distribution as compared with the past years. Variation in rainfall between 2014 and 2018 for the district ranged from 192 mm to 286 mm in July to 131 mm to 195 mm in August, and 60.4 mm to 157 mm in September (RSD, 2019). As a water-efficient plant, quinoa is considered to perform well with satisfactory yields under a rainfall of 100 to 200 mm (FAO, 2011), suggestive of the critical requirement of supplementary irrigation in absence of adequate rainfall. The average rainfall during sowing and harvesting months in the trial sites ranged from 0 to 14 mm, reflective a dry season during the study period. Our observation at the on-station seed production field at the research centre in Yusipang supports this premise that prolonged dry period (without supplementary irrigation) in quinoa sown in poor and virgin soil leads to failure in germination as well as death in some germinated seedlings. Contrary to quinoa widely acclaimed as a climate-resilient crop, resistant to adverse abiotic stresses like drought and frost (Bazile, Bertero, & Nieto, 2015; S. E. Jacobsen et al., 2005; S. E. Jacobsen et al., 2003), the crop is found highly susceptible to either failure in seed germination or death of germinated seedlings under moisture-stressed soil conditions.

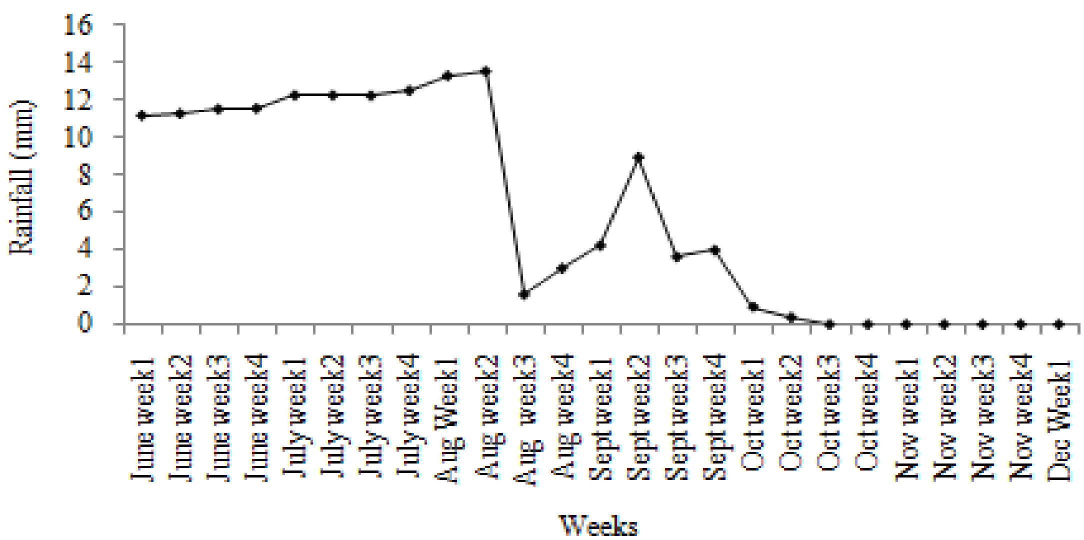

Figure 3. Variation in rainfall during the growth and development of quinoa. Source: (NCHM, 2017).

Table 3. Total monthly rainfall of Haa from the year 2014-2018.

Year	Monthly rainfall in mm												Total
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	
2014	0	0	23	29.7	133.5	101.6	194.4	195.6	127.3	19.9	0	19	844.4
2015	4	32	61	119.1	71.2	141.8	206.4	215	156.1	36.8	0	0	1042.9
2016	0	3.8	70	44.9	82.2	84.2	286.8	131.5	157.1	116.2	0	0	976.8
2017	8.1	0	67	81.3	99.8	80.7	197.8	153.4	147.4	22.1	0.7	0	858.4
2018	2.7	7.9	51	45.6	57.3	71	192.5	170.5	60.4	5.9	51.4	10	726.2

Source: (RSD, 2018)

3.4. Temperature

Daily atmospheric temperature affects seed germination and seedling rate as well as plant growth. Figure 4 shows the weekly variation in temperature of Haa. There were no huge variations in the weekly temperatures from June to September. Although the maximum temperature dropped slightly from the 3rd week of September, it climbed up (34 ^{0}C) in the first week of November. The biggest drop in minimum temperatures starts from October 2nd week. This agrees with the finding reported by (Katwal & Bazile, 2020) for cool temperate agro-ecological zones where the occurrence of frost starts as early as the second fortnight of October and continues until the first fortnight of April. They found frost damage to quinoa was evident when the crop was sown in the first fortnight of March and in August. Their preliminary observation trials on the cultivation of quinoa as a second crop after potato in the warm and cool temperate areas indicate that sowing quinoa in these areas has to be done by mid-July to escape frost damage at anthesis. Quinoa is sensitive to environmental stresses from the first anthesis to the end of flowering (Bertero & Ruiz, 2008).

Temperature and sensitivity to photoperiod are the main abiotic factors affecting germination, growth and productivity in quinoa (Christiansen, Jacobsen, & Jørgensen, 2010). To characterize the growth and development of quinoa, analysis of response to temperature and photoperiod in all developmental phases for a large number of genotypes are required (Bertero, King, & Hall, 1999). FAO (2011) reports the ideal temperature for quinoa growth as between 15 to 20 °C with the plant being able to withstand temperatures from -4 °C to 38 °C. Further, S.E. Jacobsen and Bach (1998) observed that the optimal temperature for growth of quinoa is 22 °C, and the base temperature as 3 °C. Our results indicated that while cool temperate agro-ecological conditions in Haa meet the ideal temperature requirements for quinoa, its growth and yield are negatively affected for both the varieties sown on 15 August as plants could not develop further due to the drop in both day and night temperatures starting October. This suggests that quinoa varieties sown in mid-July will not perform well in the cool temperate zones.

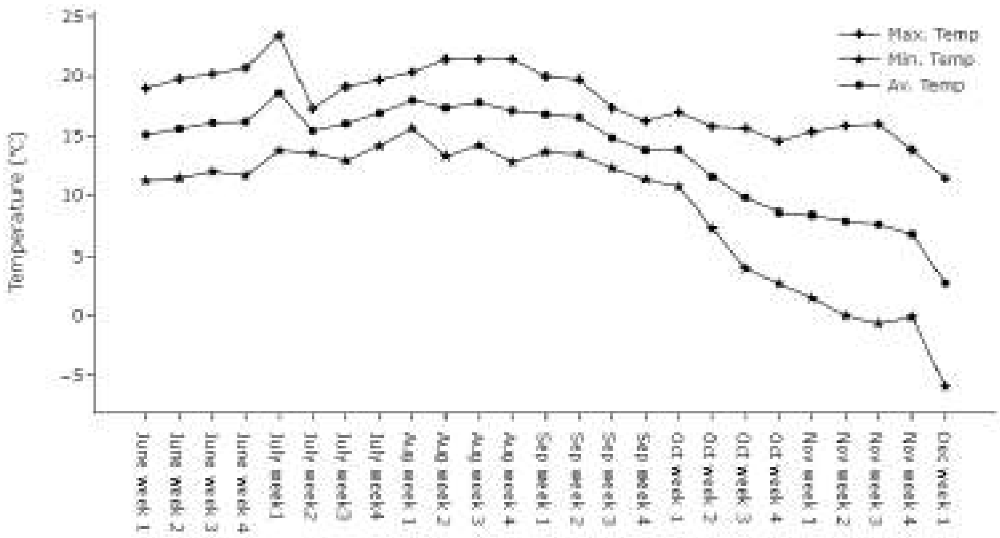

Figure 4. Temperature variation during the growing period. Source: (NCHM, 2017).

3.5. Growing degree days

To provide understanding and estimate the development of not only quinoa plant but also the emergence and development of pests and weeds in the crop, Cumulative Growing Degree Days (GDD) variation during the period of the experiment is presented in Figure 5(worked out using the following formula):

$$\mathrm{GDD} = \frac{(Tmax - Tmin) - \mathrm{Tbase}}{2}$$

Tbase for quinoa is taken as 3 °C from Jacobsen & Bach (1998). With a uniform trend in GDD between June and July, the highest increase in GDD was observed from August to September and then it decreased from October at a much faster rate.

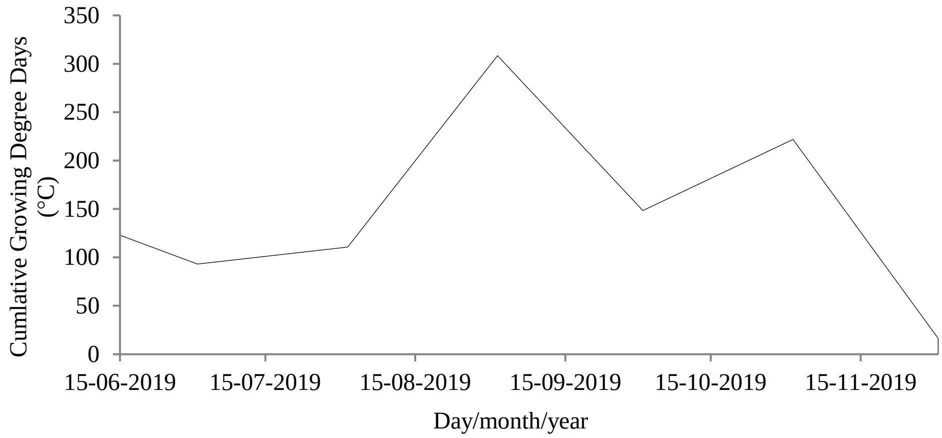

Figure 5. Variation of cumulative growing degree days. Data source: (NCHM, 2017).

4. Conclusion

It is clear that sowing dates have a significant effect on quinoa growth and productivity which is also influenced by climate parameters, such as temperature, photoperiod and rainfall, including and some biotic factors. Germination and growth were also affected due to severe weed pressure and leaf miner infestation in all the sowing treatments. The first treatment was damaged by leaf miner despite spraying neem oil in an attempt to control the pest organically. This also suggests that neem oil may not be effective for managing leaf miner infestation in quinoa. Remaining treatments were saved by spraying synthetic insecticide. Weed infestation was high despite providing three hands weeding and hoeing during the critical growth stage. The study also demonstrates that yield of quinoa sown on 15 July is significantly higher than the national average yield and suggests potential in increasing yield further through provision of good management practices, such as supplementary irrigation, adequate application of quality farm manures, and weed and pest control measures. However, in actual practice, growers apply adequate quantity of chemical fertilizer (Suphala) in potato crop following which quinoa can effectively assimilate the residual manures and fertilizers.

Amarilla variety returned the highest grain yield at 409.30 kg/acre with the corresponding highest plant height of 77.31 cm for 15th-July sowing. Similarly, DOA's highest yield was 369.63 kg/acre with a plant height of 56.60 cm for the same sowing date of 15th July. The least yield and plant height were obtained for quinoa planted on 15 August. As far as the study is concerned, it can be suggested that quinoa variety AM when sown later than July 15 in Haa results in the plants being subjected to extremely cold winter temperatures during its critical growth stage, and quinoa farming as a second crop after potato harvest is not possible. However, DOA variety could be selected for late July sowing due to its shorter growing duration as compared to AM. Currently, potato is harvested generally towards the last week of July, so this variety fits well into the crop rotation cycle.

Quinoa as a proven complete nutritious, healthy and highly recommended food is being promoted rapidly across the country by the Department of Agriculture as a climate-resilient and nutrient-dense crop to diversify existing traditional cropping system. This study has attempted to complement this initiative of the department by providing opportunities in making necessary adjustments into sowing time, identifying suitable varieties and crop management practices to help fit the crop into as many growing environments and the farming systems as possible. Quinoa research and development intervention continue to be an important activity in the nation's 12 Five Year Plan, and the findings of this study will partly help in these development objectives.

Acknowledgement

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Assessment of Performance and Oil Recovery (%) in Existing Groundnut (*Arachis Hypogaea* L.) Accessions

Kinzang Thinley[k], Namgay Wangdi[l], Tshering Choden[k], Tashi Dema[m]

ABSTRACT

Groundnut cultivation in Bhutan is predominantly restricted to few farmers for its kernels whereby oil is usually not extracted. The rich agro-ecological diversity hosts a multitude of groundnut accessions that have not been studied so far. Based on geographical location, seed colour, seed shape or pattern, five local accessions, namely Chalipa badam, Bartshampa white badam, Yangbrangpa red badam, Yangbrangpa white and purple badam, and Nanongpa badam were identified in eastern Bhutan. A multi-location trial using RCB design was conducted across four agro-ecologically diverse locations namely; ARDC-Wengkhar, ARDSC-Lingmethang, ARDC-Samtenling, and ARDSC-Tsirang in April 2019. Days to 50 % flowering, plant height, number of pods per plant, thousand-seed weight, shelling %, yield, and oil recovery % were assessed. The yield performance of all five accessions was poor in the low/hot environment as compared to that in the mid/cool environment where all accessions, except Nanongpa badam which was marginally low yielding, gave satisfactory and similar yields. Correlation between the number of pods per plant and yield showed a high positive relationship (r = 0.75) whereas that between plant height and yield was negative (r=-0.57).

Keywords: *Groundnut; Accessions; Numbers of pods; Yield; Oil recovery %*

1. Introduction

Groundnut (*Arachis hypogaea* Linn.) is an important crop among oilseeds belonging to the family Leguminosae which is widely grown as an annual crop in the tropics and subtropics for its edible seeds and oil production around the world (Anjani & Khabiruddin, 2017; Ansah, Yaccub, & Rahman, 2017). Groundnut is often referred to as wonder nut, poor men's cashew nut (Madhusudhana, 2013; Ramapadmaja & Rao, 2019), peanut or gobber (Nasar, Qiang, & Alam, 2018), earthnut (Lawan, Ali, Abubakar, & Muhammad, 2015), monkey nut, and manila nut (Kokkiripati, Rai, Marker, & Veraja, 2015) owing to the development of fruits/pods below the ground (Akhtar et al., 2014; Yol, Furat, Upadhyaya, & Uzun, 2018; Zaman et al., 2011). Due to the rich digestible proteins, the crop is considered as 'king' of oil crops which is being produced from edible seeds and sometimes is referred to as Arachis oil and peanut oil (Aluyor, Aluyor, & Ozigagu, 2009; Konate et al., 2019; Madhusudhana, 2013; Ramapadmaja & Rao, 2019).

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Groundnut kernels contain 40-56 % edible oil, 20-30 % protein, 10-20 % carbohydrate, vitamins such as E, K and B (Kassa, Yeboah, & Bezabih, 2008; Savage G.P. & Keenan, 1994; Toomer, 2018), and dietary minerals such as niacin, calcium, magnesium, phosphorus, zinc, iron, riboflavin, thiamine, and potassium (Dean, Hendrix, Holbrook, & Sanders, 2009; Savage G.P. & Keenan, 1994). Groundnut oil is a rich source of plant sterol especially b-sitosterol, which is known to have anti-cancer properties and reduces cholesterol levels by 10-15% by inhibiting cholesterol absorption (Shasidhar et al., 2017). The cake obtained after oil extraction can be used in animal feed (Savage G.P. & Keenan, 1994) and it also improves soil fertility and productivity by fixing atmospheric nitrogen (Akhtar et al., 2014; Nautiyal et al., 2011). Globally, 37.1 million metric tons (MT) of groundnut production was reported from 26.4 million hectares with an average productivity of 1.4 MT per acre (Saravanan, Rajkala, & Alagukannan, 2018).

In Bhutan, groundnut is predominantly a minor crop grown by some farmers across the country. It may be grown for various purposes, such as for the sale of its kernels as a snack in the local market or for its above-ground biomass as nutritious fodder for animals. It is seldom grown for extracting its oil. After all, the availability of cheap imported refined vegetable oils of all kinds in the market leaves hardly any reason to go for local groundnut oil production which is not economical given the small-scale and scattered nature of the producers.

Although groundnut is hardly a crop of any economic importance in Bhutan, the rich diversity found within the crop is noteworthy due to the diverse agro-ecological environments. Genetically the crop may be the same but the specific local environment where it is grown can impart special traits over time giving each accession unique characteristics that contribute to their resilience and adaptation. In eastern Bhutan, five such local accessions of groundnut have been identified based on colour, the shape of the kernel, kernel size or colour patterns, and more importantly, based on where each accession is grown predominantly. Figure 1 shows the picture of each accession based on the above description.

Chalipa badam
Bartshampa white badam
Yabrangpa red badam
Yabrangpa white and purple badam
Nanongpa badam

Figure 1. Seed of groundnut accession studied.

To describe each accession briefly, Chalipa badam is typically found growing in Chali village, which features moderate pod beak and pod constriction with deep purplish-red colour seeds. Bartshampa white badam accession has off-white seed colour, slight pod beak, slight constriction of the pod, and moderate reticulation of the pod. In Yabrang village under Tashigang Dzongkhag, there are two groundnut accessions; one has dark red seed colour with moderate pod beak, slight pod constriction, and slight pod reticulation which is classified as Yabrangpa red badam, and the other has variegated striped purple and off-white seed patterns, slight pod beak, none pod constriction and slight reticulation of the pod (Yabrangpa white and purple). Nanongpa badam has a very prominent pod beak, a slight constriction of the pod, a prominent reticulation of the pod, and an elongated light tan seed.

Notwithstanding such diversity, there are hardly any studies done on groundnuts in Bhutan, leave alone the local accessions described above. Studying and documenting our local accessions/ germplasm is important as the first step towards any long-term development strategy of commodities.

This study was therefore done to assess the performance and adaptability of the five groundnut accessions in different agro-ecological environments of Bhutan.

2. Materials and methods

The experiment was conducted in four locations across the country under open field conditions namely; 1) Agriculture Research and Development Center (ARDC)-Wengkhar; 2) Agriculture Research and Development Sub-center (ARDSC)-Lingmethang; 3) ARDSC-Tsirang; 4) ARDC-Samtenling. The five groundnut accessions tested were: (T1 = Chalipa badam, T2 = Bartshampa white badam, T3 = Yangbrangpa red badam, T4 = Yangbranpa white and purple badam, T5 = Nanongpa badam). The multi-location trial was started in April 2019 using a randomized complete block design (RCBD) with three replications. Each experimental plot measured 4.5 m^2 with 0.5 m between treatments and 1 m alley between the blocks. The groundnut kernels were planted at the spacing of 30 cm between rows and 10 cm between plants. The agronomic practices inclusive of irrigation, weeding and earthing-up were followed as per the package of practices (PoP) of groundnut. A total of 10 plants (samples) from each plot were randomly tagged through a simple random sampling method for observation and assessment. The agronomic traits such as days to 50% flowering, plant height at maturity (cm), numbers of pods per plant, shelling percentage (%), yield (MT per acre), thousand-seed weight (g) and oil recovery percentage (%) were evaluated. The days to 50% flower formation were counted by days from sowing to date when 50% of the plants produced flowers in each plot. The heights of the 10 representative samples in each plot from ground level to the plants' growing tips were measured in centimeters to record plant height. The number of pods per plant (NPP) was recorded as the average number of pods from 15 randomly sampled plants. The pod yield was measured by weighing the dried pods from each plot and was recorded in kilograms per plot. Later, the measured yield was extrapolated to determine pod yield per acre of land by adopting the succeeding formula:

1. Yield (MT/ac) = [(Average yield of plot (kg) x 4000 m^2 x $_{MC\ (ad)}$/ (Plot size (m^2)] …(i)

To determine shelling percentage, pods of whole plots were shelled from seed and then converted into % by adopting the following formula (Raza et al., 2017; Jeyaramraja and Woldesenbet, 2014) below:

2. Shelling percentage (%) = (Total plot yield-yield after shelling/Total yield) x 100...…(ii)

At physiological maturity, 1000 kernels from tagged plants were assembled and their weights in grams measured.

The crude oil extraction was carried out only in ARDC-Wengkhar using a Japanese mini oil compressor (product model number d5 Rinka Ikuto, DK-119). Before extraction, the seeds were thoroughly sun-dried and peeled manually. A total of 0.5 kg of seeds from each plot was weighed for oil extraction. The determination of the oil recovery method was adopted from the study of Nkafamiya, Maina, Osemeahon, and Modibbo (2010) in which expelled oil was measured using a measuring cylinder and the unit was expressed in millilitres (ml).

The percentage (%) yield of oil was calculated using the equation (Pardeshi, 2019; Rodas & Cruz, 2017) below:

3. Oil recovery (%) = (Weight of oil (ml)/Weight of sample (g)) x 100………..... (iii)

The analysis of data was done using the statistical tool STAR and Stata software. The data from the four locations/sites were combined to examine location-accession interaction, main effects, and simple main effects. The Shapiro-Wilk test for normality of residuals and Bartlett's test for homogeneity of variance were carried out to check for violations of assumptions required for ANOVA. The Pearson correlation coefficient test was performed to determine the correlation matrix between the two variables. A probability level of $P < 0.05$ was considered significant.

3. Results and Discussions

3.1. Days to 50% flowering

Flowering traits in groundnut are indicative of the maturity period of genotype (Upadhyaya & Nigam, 1994; Upadhyaya, Reddy, Gowda, & Singh, 2006) and play a significant role in all seed crops (Kaba, Kumaga, & Ofori, 2014). The late-maturing groundnut genotypes generally took more days (> 30 days after planting) to reach 50% flowering (Oteng-Frimpong, Konlan, & Denwar, 2017; Poehlman & Sleper, 1995). The groundnut accessions that accumulate early flowers have the potential for developing early maturing and short-duration cultivars (Nigam & Aruna, 2008).

Combined analysis showed no significant site-accession interaction effect (P=0.40), no significant accession effect (P=0.90) but highly significant site effect (P=0.000).

Table 3. Mean number of days to 50% flowering of groundnut accessions across sites.

Location	**Mean**	**Std. Err.**	**95% CI**	
Wengkhar	48.60	0.77	47.02	50.18
Lingmethang	36.00	0.77	34.42	37.58
Tsirang	48.40	0.77	46.82	49.98
Samtenling	38.40	0.77	36.82	39.98

The accessions took about 11 days more to attain 50% flowering in the mid/cool environments (Wengkhar and Tsirang) than at low/hot environments (Lingmethang and Samtenling). The variation in 50% flowering days may be due to responses of each genotype to the growing environment (Oteng-Frimpong et al., 2017; Yol et al., 2018), genetic makeup (Ishag, 2000), and its taxonomic differences (Gupta, 2012) and growing temperature (Craufurd et al., 2000).

3.2. Plant height

The overall height of the accessions averaged across all four sites was 104.37±39.68 cm. The mean height of the accessions observed across the four sites showed that height from the Lingmethang trial was observed about 2.7 times more than that of Wengkhar or Tsirang and about 1.4 times of Samtenling as shown in Figure 2. This might be attributed due to the higher temperature prevalent there favouring rapid vegetative growth, but the same was not true at Samtenling where the height was only slightly taller than at Wengkhar/Tsirang, and one might suspect other reasons at play, such as over-fertilized plots.

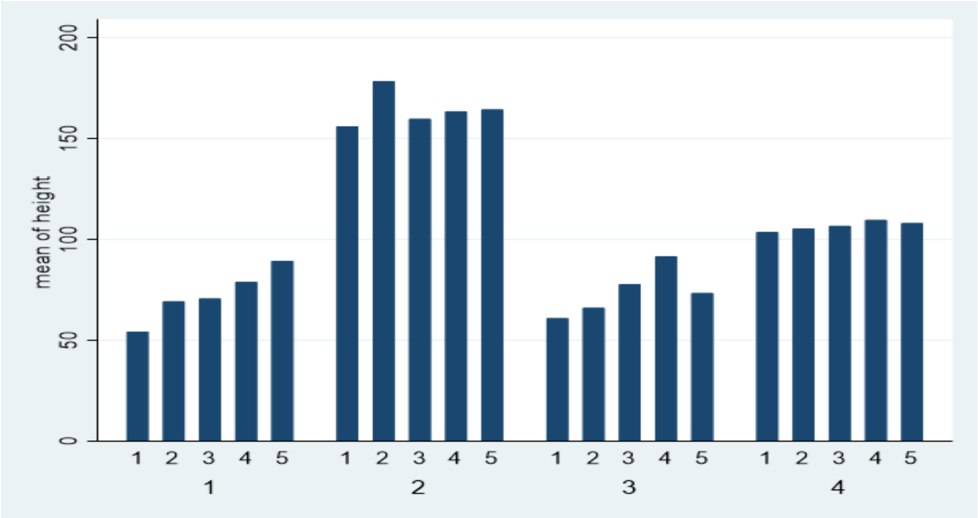


Figure 2. Mean height (cm) of accessions across the sites.

The correlation between height and yield was negatively related (-0.57) and it was highly significant (*P*=0.000). In general, it is a common fact that when plants partition a lot of energy towards vegetative growth, assimilation in the grains or fruits is reduced.

Overall, the accession Yabrangpa White and Purple badam exhibited the tallest height among other accessions (114.05 cm) and Chalipa badam with the shortest height (95.38 cm). Even though the combined analysis showed a significant effect of accession (*P*=0.000), this question cannot be answered straightforwardly since site-accession interaction was also significant (*P*=0.01). This variation in plant height could be attributed due to different growth rates of accessions (Raza et al., 2017), the response of accessions to the environment and the genetic makeup of the genotype (Kokkiripati et al., 2015).

3.3. Number of pods per plant

The number of pods per plant is one factor that determined the yield of the groundnut (Yol et al., 2018). The genotypes having a higher number of pods per plant provide an opportunity for improving seed yield in groundnut (Luz, Santos, & Filho, 2015). A scatter plot analysis of yield on the number of pods revealed there was an obvious positive linear relationship and this was also consistent with a high correlation coefficient of 0.75 (*P*=0.000).

3.4. Shelling recovery (%)

Shelling recovery percentage (%) is regarded as an index of the percentage of grains or seeds (Dapaah, 2014) and is a vital factor for selecting accessions in groundnut breeding (Anothai et al., 2008). Table 2 shows the shelling recovery percentage of the accessions across the four sites. The site-accession interaction was not significant (*P*=0.20), allowing for the main effect comparison. There was no significant difference amongst the accessions while the effect of location was significant (*P*=0.01).

Table 4. Mean shelling % of the accessions across the sites.

Treatment	**Experimental sites**				**Average shelling %**
	Wengkhar	**L/thang**	**Tsirang**	**S/ling**	
Chalipa badam	45.24	27.05	38.51	28.60	34.85
Bartshampa white badam	39.11	29.16	29.44	36.39	33.52
Yabrangpa red badam	32.22	28.42	31.06	33.06	31.19
Yabrangpa white and purple badam	38.53	28.77	34.53	27.17	32.25
Nanongpa badam	33.01	28.35	46.00	42.16	37.38
Average	37.62	28.35	35.91	33.48	33.84

The mean shelling recovery of the five accessions was highest for Wengkhar (37.6%) and lowest for Lingmethang (28.3%). The highest shelling recovery might be due to genotypic x environment interactions (Minimol, Datke, Deshmukh, & Satpute, 2001; Raza et al., 2017) and asserted due to the presence of calcium in the soil (Bucheyeki, 2008).

3.5. Thousand seed weight

The 1000 seed weight differed significantly between the various locations (*P*=0.000), with the S/ling mean being the highest and Lingmethang lowest as shown in Table 3. Within the accessions, except for a significant difference between Chalipa badam and Bartsampa badam and between Chalipa badam and Yangbrangpa white and purple badam, there were no significant differences between other pairs. This might be due to the yielding of larger seed size, production environment, and cultural practices (Yol et al., 2018).

Table 5. Mean thousand seed weight of the accessions across the locations.

Treatment	**Experimental sites**				**Average (g)**
	Wengkhar	**L/thang**	**Tsirang**	**S/ling**	
Chalipa badam	633.33	319.33	735.00	800.00	621.92
Bartshampa white badam	566.67	386.00	536.67	646.67	534.00
Yabrangpa red badam	583.33	340.33	696.67	736.67	589.25
Yabrangpa white and purple badam	516.67	273.67	631.67	673.33	523.83
Nanongpa badam	550.00	355.33	613.33	666.67	546.33
Average	**570.00**	**334.93**	**642.67**	**704.67**	**563.07**

3.6. Yield

Individual analysis of variances at each site revealed no significant difference in yield among the accessions. The accessions performed quite well at Tsirang followed by Wengkhar. The yield performance might have influenced due to cumulative performance of the genotype's response to environments, the genetic make-up of the specific accessions (Kokkiripati et al., 2015; Samaha, 2019), production environment, cultural practices (Yol et al., 2018) and air and soil temperature as groundnut flowers develop aerially and pods in the soil (Kumar, Singh, & Boote, 2012). However, the yield observed at S/ling was in particular very poor and that at L/thang was only slightly better but overall, it suggests that the accessions are not adapted to low/hot environments.

Table 6. Mean yield (MT/acre) of the five accessions over four sites with *P*-values and cv%.

Treatment	**Wengkhar**	**L/thang**	**Tsirang**	**S/ling**
Chalipa badam	1.837	0.667	3.500	0.050
Bartshampa white badam	1.837	0.783	4.367	0.037
Yabrangpa red badam	1.897	0.693	4.200	0.047
Yabrangpa white and purple badam	2.160	0.377	3.833	0.047
Nanongpa badam	1.643	0.947	2.867	0.037
P-value	0.15	0.29	0.18	0.49
CV (%)	11.48	42.92	17.17	25.97

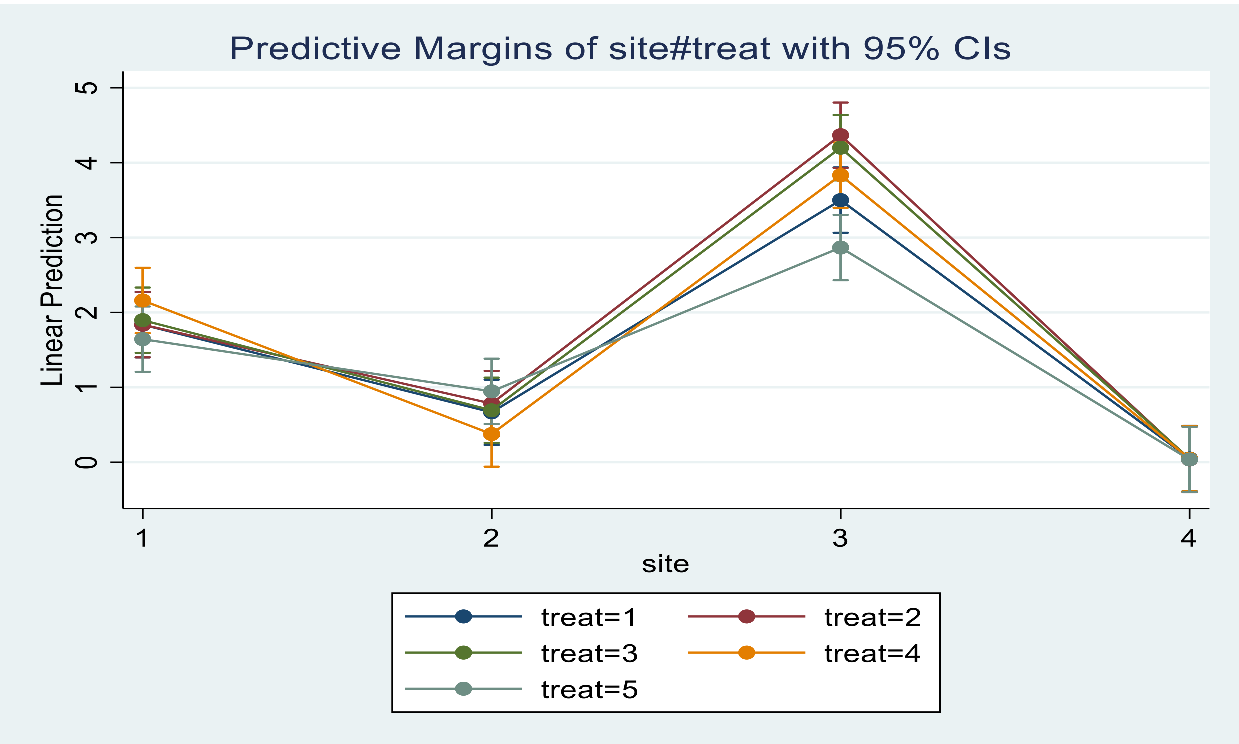


Figure 3. Predictive margins of sites.

Nonetheless, a combined analysis was performed which revealed that while site effect was highly significant (which was expected), accession effect was not and site-accession interaction was marginally significant (*P*=0.02), and this may be because all the accessions performed badly in low/hot environments while clearly showing good and marked within-accession differences at mid/cool environments as illustrated in Figure 3.

Given that the performance of the accessions is substantially poor at L/thang and S/ling, we may as well ignore them from the combined analysis so we can examine closely the performance and interaction effects at mid/cool environments where their promotion is most likely. Further, as Gomez and Gomez (1984) suggested, trial data from individual analysis of variance having cv% larger than 20% can be excluded from combined analysis.

Thus, combined analysis with L/thang and S/ling data excluded was carried out and the results showed significant effects of accession (*P*=0.03), while interaction effect disappeared as shown in Table 5.

Table 7. Combined ANOVA table.

Source	**Partial SS**	**df**	**MS**	**F**	**Prob>F**
Replication within site	1.0270267	4	0.256757	1.11	0.3849
Site	26.470412	1	26.47041	114.68	0.00000
Accession	3.0283866	4	0.757097	3.28	0.0383
site#accession	1.6838534	4	0.420963	1.82	0.1736
Residual	3.6932398	16	0.230827		
Total	**35.902919**	**29**	**1.238032**		

Since the site-accession interaction effect was not significant (P=0.17), we look at the main effects only and ignore the simple effects, i.e., we are not concerned with the differences among the accessions at respective sites even though it may be tempting as Figure 4 suggests. At best we can see that Yangbrangpa white and purple badam which yielded the highest at Wengkhar became the third-best performer at Tsirang, or Bartsampa white badam which was an average performer at Wengkhar taking the top spot at Tsirang. These are evidence of interaction occurring but as the test showed, it may be due to chance.

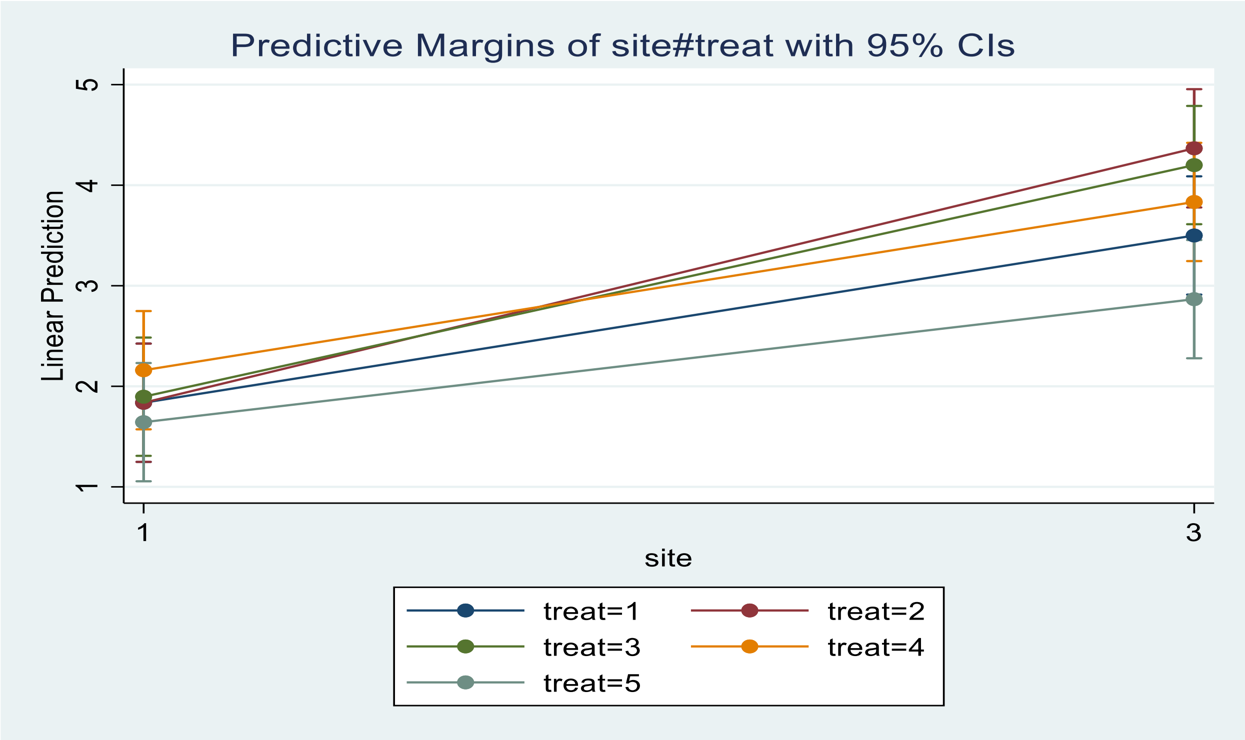


Figure 4. Predictive margins of sites.

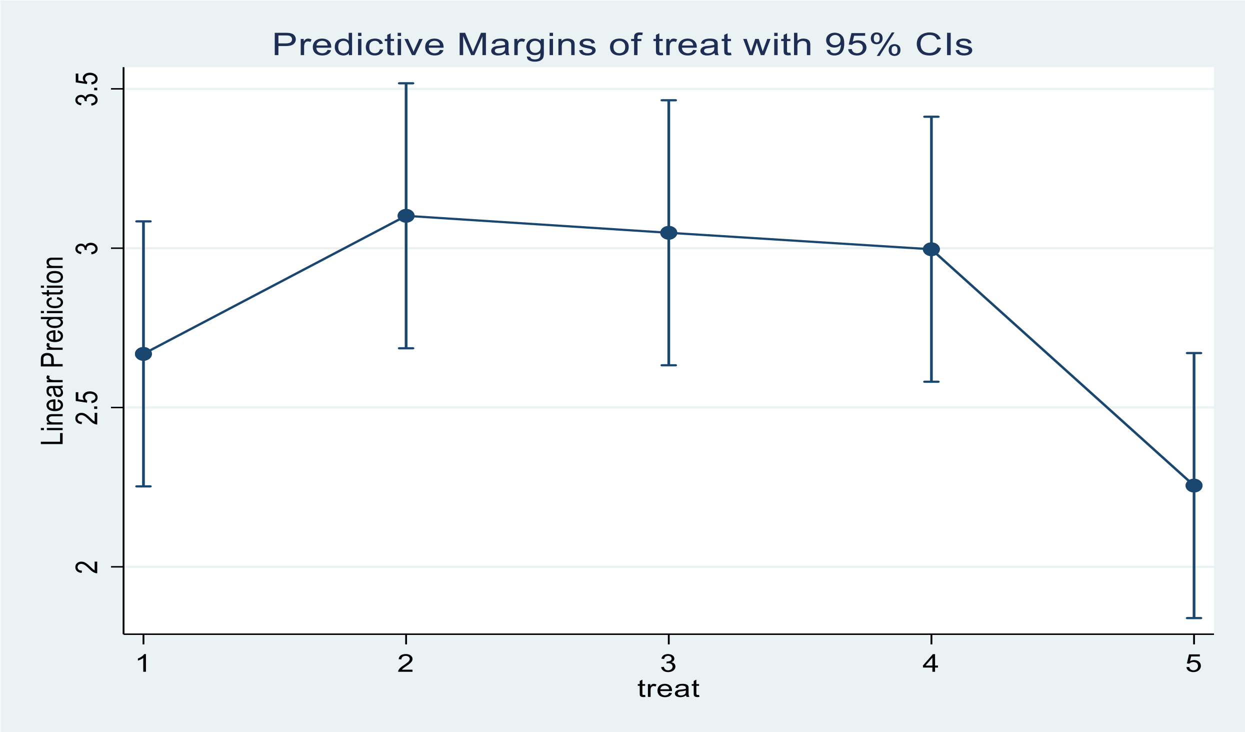


Figure 5. Predictive margins with 95% confidence intervals.

Coming back to the main effects of accession which was marginally significant (*P*=0.03), we looked at which pairwise differences were significant, using the Tukey-adjusted method. It was between Bartshampa badam p/w badam and Nanongpa badam (*P*=0.05), with all other pairs not significant as shown in Figure 5.

3.7. Oil recovery (%)

The mean groundnut oil recovery percent ranging from 18.6 to 30.73% signifies its suitability for commercial production (Anyasor et al., 2009). The analysis of variance showed no significant difference (*P*=0.53) in oil recovery percentage among the accessions. However, the genotype Chalipa badam revealed maximum oil recovery with 30.73% followed by Yangbrangpa white badam (25.33%) and lowest in Bartshampa white badam with 18.6% as shown in Table 6. The probability matrix showed that Chalipa badam has the potential to yield 553.29 litres of oil per acre followed by Yabrangpa white and purple badam (528.90 litre/acre), however, there are no statistically significant differences (*P*=0.53) between accessions. The highest oil content might be attributed to its agronomic traits, geographical locations (Kokkiripati et al., 2015), seed maturity, growing season, growth condition (Asibuo et al., 2008; Zahran & Tawfeuk, 2019), influence by climate status and temperature (Rodas & Cruz, 2017; Samaha, 2019). The result was similar to the standard groundnut oil content (32%) USDA 2008-2009, as cited in Akhtar et al. (2014).

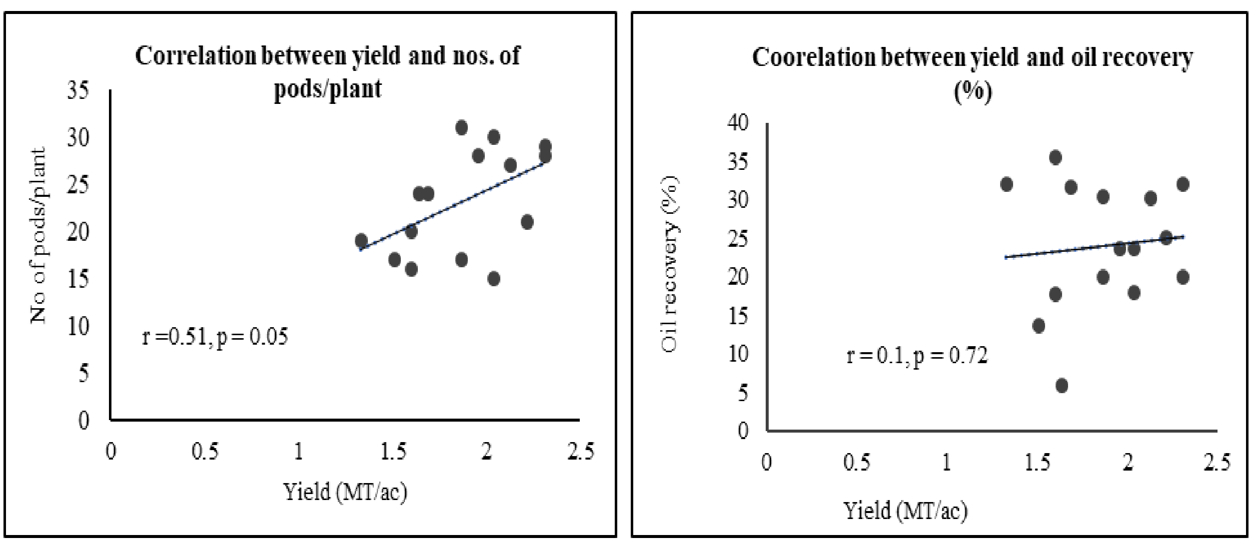
Table 8. Oil recovery (%) among five groundnut accessions.

Groundnut genotypes	Oil (ml/0.5 kg)	Oil content (l/ac)	Oil recovery (%)
Chalipa badam	153.67	553.29	30.73
Bartshampa white badam	93.00	342.00	18.60
Yangbrangpa white badam	126.67	504.35	25.33
Yangbrangpa white and purple badam	123.00	528.90	24.60
Nanongpa badam	102.67	329.17	20.53
***P*-value**	0.53	0.35	0.53
Standard error	36.23	133.57	7.25
SD	40.87	167.5	8.17
CV (%)	37.04	36.23	37.04
Mean	119.80	451.54	23.96

CV = Coefficient of variation, SD = Standard deviation, significant at P<0.05, means within columns followed by the different superscript letters indicates significance among the treatment.

3.8. Correlation matrix between yield, numbers of pods, and oil recovery (%)

The correlation coefficient between yield and oil recovery percentage showed a weak positive correlation (r=0.1) and was not statistically significant (*P*=0.72) as shown in Figure 6. It indicated that oil recovery percentage increases with an increase in groundnut yield. The correlation matrix between numbers of pods per plant and yield showed statistically significant (*P*=0.05) with a moderate positive correlation (r=0.51) as revealed in Figure 6. Similarly, a study by Shah et al. (1993) reported that yield was positively correlated with the number of pods per plant.



4.Figure 6. Correlation matrix of studied parameters in the native groundnut accessions.

Based on this one season cropping trial, in general, the groundnut accessions attained 50% flowering earlier or grew taller in the low/hot environments represented by Lingmethang-Samtenling type of environments than in mid/cool environments represented by Wengkhag-Tsirang type of environments. However, in terms of yield performance which is perhaps the main agronomic trait of interest all the five accessions performed poorly in the low/hot as compared to mid/cool environments. Closer examination within the mid/cool environment showed that all accessions, except Nanongpa badam which was marginally low yielding, gave similar yields. The oil recovery assessment, which was done only for the Wengkhar site showed promising results, especially for Chalipa badam.

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Determination of Post-harvest Losses of Quinoa at Different Stages of Handling at Saling Gewog, Mongar District

Karma Dorji[n], Kinley Wangmo[o], Dechen Tshering[n]

ABSTRACT

Quinoa (Chenopodium quinoa) is a nutrient-dense cereal crop that has been introduced in Bhutan in recent years. There is no data in Bhutan on post-harvest handling losses of quinoa, and literature from other countries are limited. This study was conducted in Saling Gewog under Mongar District to assess the losses of quinoa at different post-harvest handling stages like field drying after harvest, threshing, and drying after threshing and also to determine weight losses during storage and de-husking. Field drying loss of 1.4% and threshing loss of 2.3% with a total handling loss of 3.7% was recorded in Lingmethang. Field drying loss was 6.5% and threshing loss was 3.4% with a total handling loss of 9.9% in Yongkola while in Tzenzebi, the total handling losses increased to 14.7% including field drying loss of 7.1% and threshing loss of 7.6% with a significant difference in losses between these locations with a P-value < 0.000 for both the field drying after harvest and for threshing. Weight losses during drying were 3.4%, 4.7% and 5% for quinoa samples from Lingmethang, Tzenzebi and Yongkola respectively (P < 0.005). Losses during storage and de-husking of quinoa were determined only for the quinoa samples from Lingmethang. Weight loss at the end of three months of storage was very minimal at 0.2%. A large quantity of quinoa (19.2 %) was lost as husk during the de-husking process. Interventions from the relevant agencies are needed to improve the techniques on field drying, threshing and storage practices to reduce post-harvest losses of quinoa in Bhutan.

Keywords: *Quinoa; Post-harvest losses; Weight loss*

1. Introduction

Quinoa (*Chenopodium quinoa*) is an annual herbaceous plant belonging to the family Amaranthaceae. It is a pseudo-cereal crop with a broadleaf plant and starchy dicotyledonous seeds (Sharma, Chandra, Dwivedi, & Parturkar, 2015). Its origin is believed to be from the Central Andes in South America (Martínez, Fuentes, & Bazile, 2015). Quinoa is said to be a nutrient-dense cereal crop and is promoted as high nutritional food in many countries around the world (Gamboa, Van den Broeck, & Maertens, 2018). Quinoa has been recognized by the Food and Agriculture Organization (FAO) as a strategic crop that can contribute to global food security because of its

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high nutritional quality, genetic variability, adaptability to adverse climate and soil conditions, and low production cost (FAO, 2011; Parra, Rohringer, Garcia-Molano, & Ortiz-Gonzalez, 2020).

In Bhutan, quinoa was introduced in 2015 from Peru by the Department of Agriculture with support from FAO with an objective to diversify crops and the current cropping systems, and to enhance the food and nutritional security of the Bhutanese people (Katwal, Wangdi and Giri, 2019). In Bhutan, quinoa production was estimated to be 9 tons from 70 acres of harvested area with an average yield of 132 kg/acre in 2017 (DoA, 2017).

The maturity of quinoa depends on the variety and altitude at which it is grown. In high altitude areas (above 1200 masl), the maturity takes 120 to 150 days after sowing while 170-180 days are required for longer duration varieties to mature. In the warm areas below 1200 masl, the quinoa crop matures much faster (Katwal, 2018). Quinoa crops are sun-dried or made into bundles and hung by the ceilings immediately after harvest for drying. It should be dried properly for easy threshing. Well-dried quinoa grains can be put in polypropylene bags or plastic bins and stored in a cool and dry room. Milling or de-husking can be done manually using a locally made de-husking device used in villages to de-husk rice and millet or mechanically using a de-husking machine. Only the well-dried quinoa should be used for milling (Katwal, 2018).

Any nation needs to carry out studies on post-harvest losses of crops to determine how much food is lost after harvest before reaching the consumers and at what stages the major losses occur for the particular food crops. Accurate data on post-harvest losses of crops at different stages of handling can be beneficial for policymakers and relevant organizations to make appropriate interventions to help reduce food losses. This would ensure that the food that has been produced after putting so many inputs reaches the market to feed the growing global population.

According to the Food and Agriculture Organization (2015) of the United Nations, 1.5 billion tons of food are wasted or lost per year. The food losses and wastes amount to roughly US$680 billion in industrialized nations and US$310 billion in developing countries (FAO, 2015). It was revealed that post-harvest loss of cereal in Sub-Saharan Africa ranged between 5-40 % with an estimated value of around $4 billion while losses of cereal crops in the developing countries are estimated to be as high as 25% of the total production (Zorya et al., 2011).

Since its introduction in the country, no studies have been conducted on post-harvest losses of quinoa and currently, no data are available on its losses at the different stages of post-harvest handling operations. Even at the global level, the reports and data on post-harvest losses of quinoa at different stages of handling are either too little or not available. This study was conducted at Saling Gewog under Mongar district to determine post-harvest losses of quinoa at different stages of handling to compare the post-harvest losses at different locations and to assess the weight losses of quinoa during post-harvest storage.

2. Materials and Method

2.1. Survey area and data collection

The data on post-harvest losses of quinoa were collected from Tshenzebi (1400 masl), Yongkola (1500 masl), and the Agriculture Research and Development Sub-Centre (ARDSC), Lingmethang (650 masl) research field under Saling Gewog, Mongar district in the month of January 2020. The variety used in this study was 'Amarilla Saccaca' that is extensively promoted and cultivated in the Lingmethang locality. The post-harvest operations in this study were practiced as described by (Katwal, Wangdi, & Giri, 2019). The post-harvest losses of quinoa during field drying after harvest, threshing operations, and drying (after threshing) were carried out with five replications in each location. Post-harvest weight changes (post-harvest weight losses) were recorded after storage and de-husking for the quinoa samples harvested from only ARDSC Lingmethang, and no studies were carried out to determine the losses during storage and de-husking for the samples from Yongkola and Tsenzebi since there was no budget allocation to procure quinoa from farmers.

2.2. Determination of weight losses during field drying after harvest

Quinoa plants were harvested from 2 × 2 m^2 plots and dried on a tarpaulin sheet for four consecutive days within the field in the sun. At the end of the drying period, the fallen quinoa grains were collected from tarpaulin and weighed. The sum of weights of grains fallen on the tarpaulin during drying and grains that did not fall during the drying operation constituted the total grain weight of the sample. This procedure was replicated five times in each location. The field-drying losses were calculated using the formula:

$$Field\ drying\ loss\ (\%) = \frac{Weight\ of\ fallen\ grains}{Total\ weight\ of\ grains}\ x\ 100 \quad (1)$$

2.3. Determination of weight losses during threshing operation

A tarpaulin sheet was spread around the normal threshing area and grains that fell outside the threshing area were collected and weighed after threshing. The sum of weights of grains from within and outside the threshing areas constituted the total grain weight of the sample. The threshing losses were calculated using the formula:

$$Threshing\ losses\ (\%) = \frac{Weight\ of\ grains\ fallen\ outside\ of\ the\ normal\ threshing\ area}{Total\ weight\ of\ grains}\ x\ 100 \quad (2)$$

2.4. Determination of weight (moisture) losses during drying after threshing

The threshed quinoa samples were weighed and dried in the sun for two consecutive days before storage. After the completion of drying, the samples were again weighed and the difference in weight after drying was calculated using the formula:

$$Weight\ loss\ (\%)\ during\ drying = \frac{Weight\ before\ drying - Weight\ after\ drying}{Weight\ before\ drying} x100 \qquad (3)$$

2.5. Determination of weight losses during storage

At Lingmethang, five kilograms of dried quinoa were stored at ambient temperature store for a period of three months and changes in weight of sample were recorded monthly. The storage trial consisted of five replications. The difference in weight during each storage month was calculated as follows:

$$Weight\ loss\ (\%)\ during\ storage = \frac{Initial\ weight\ of\ grains - Final\ weight\ of\ grains}{Initial\ weight\ of\ grains} x100 \quad (4)$$

2.6. Determination of weight loss during milling (de-husking)

Five samples of dried and stored quinoa with five replicates were weighed before and after de-husking to find the differences in weight. De-husking was carried out using a quinoa de-husking machine. The weight loss was calculated as follows:

$$Weight\ loss\ (\%) after\ de-husking = \frac{Weight\ before\ de-husking - Weight\ after\ de-husking}{Weight\ before\ de-husking} x100 \qquad (5)$$

2.7. Data analysis

Microsoft Excel was used for basic calculations and plotting graphs. The data were analyzed using SPSS software version 21.0. The recorded data were analyzed by one-way ANOVA and the significance of treatment means were compared using Duncan's test or independent samples *t*-test ($P < 0.05$).

3. Results and Discussion

At the Agriculture Research and Development Sub-Centre, Lingmethang, the field drying and threshing losses of quinoa were 1.4 % and 2.3 %, respectively with a total handling loss of 3.7 % (Table 1). The total handling losses were 9.9 % at Yongkola (field drying- 6.5 % and threshing- 3.4 %) while the total handling losses were 14.7 % at Tsenzebi (field drying-7.1 % and threshing- 7.6 %). The average field drying and threshing losses from the above three places were 5.0 % and 4.4%, respectively that constituted a total handling loss of 9.4%. The weight losses during drying after threshing were 3.4 %, 5 % and 4.7 % for quinoa from Lingmethang, Yongkola and Tsenzebi respectively (Table 1).

The lowest post-harvest losses recorded from ARDSC Lingmethang was assumed to be due to good production and post-harvest management practices, while higher losses at farmers' field in

Yongkola and Tsenzebi could be due to the lack of or poor technical know-how on post-production handling and management operations. The higher losses in these areas could also be due to the harvest of quinoa before the maturity stage since farmers generally have minimal knowledge of maturity indices of newly introduced crops. The differences in altitude of these places could have resulted in different maturing stages of crop and contributed to the difference in losses during the post-harvest operations (Katwal, 2018).

Table 1. Losses (%) of quinoa at different post-harvest handling stages by locations.

Locations	Weight losses (%)		
	Field drying (after harvest)	**Threshing**	**Drying (after threshing)**
Lingmethang	1.4 ± 0.0^{c}	2.3 ± 0.1^{c}	3.4 ± 0.4^{b}
Yongkola/Thridangbi	6.5 ± 0.0^{b}	3.4 ± 0.1^{b}	5.0 ± 0.3^{a}
Tsenzebi	7.1 ± 0.2^{a}	7.6 ± 0.3^{a}	4.7 ± 0.2^{a}
Mean	5.0	4.4	4.4
P-value	0.000	0.000	0.005

Mean values within the columns with different superscripts are significantly different between locations for each post-harvest operation at $P < 0.05$ by Duncan's test (mean ± standard error, n=5).

Table 2. Field drying and threshing losses of quinoa at Lingmethang.

Post-harvest operations	Weight loss (%)
Field-drying	1.4±0.0
Threshing	2.3±0.7
t (8)	-10.8
P-value	0.000134

Independent samples *t*-test by SPSS version 21 (n = 5, mean ± standard error).

There was a statistically significant difference ($P = 0.000134$) between field drying and threshing losses of quinoa harvested in Lingmethang (Table 2). In Lingmethang, weight loss (change in weight) during storage for three months was very minimal at 0.2 % while the change in weight during drying was slightly higher at 3.4%. The major weight loss of quinoa occurred during de-husking with 19.2 % as shown in Table 3. The de-husking data indicates that 19.2 % of quinoa is lost as husk and other associated waste during de-husking. Storage and de-husking trials were not carried out for the samples from Yongkola and Tsenzebi.

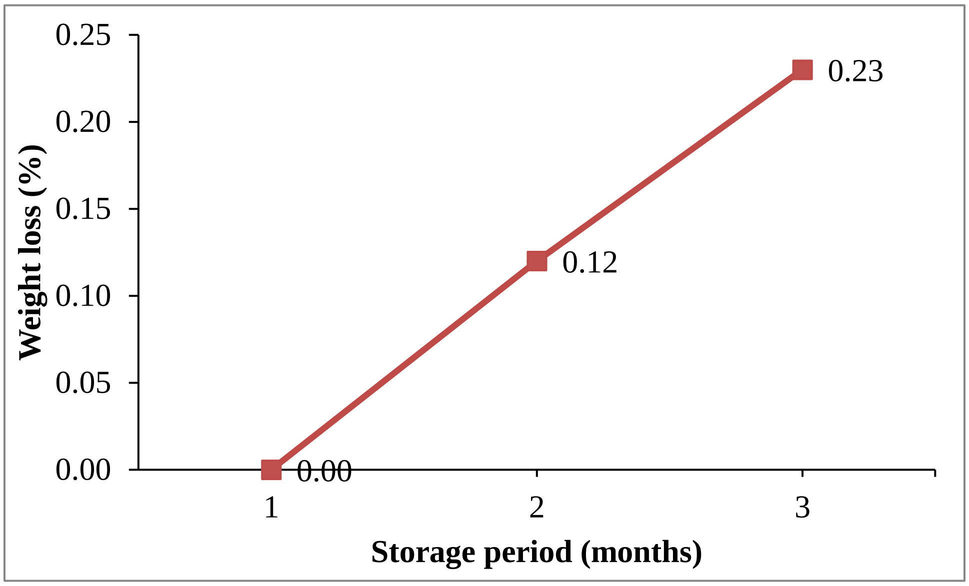
The change in weight during storage was very small as shown in Figure 1. No weight change was recorded until one month after storage, and it reached just 0.12 % and 0.23 % after storing for two and three months respectively.

Table 3. Weight loss (%) during different post-harvest handling operations for the quinoa at ARDSC, Lingmethang.

Post-harvest operations	**Weight losses (%)**
Drying	3.4±0.4[b]
Storage	0.2±0.1[c]
De-husking	19.2±0.5[a]
p-value	< 0.000

Duncan's test for weight loss respectively by SPSS version 21 (n=5, mean ± standard error). Means in the same column with different superscripts are significantly different between post-harvest operations.

Figure 1. Quinoa weight losses (%) during the three months storage period in Lingmethang.



4. Conclusion

The quantity of post-harvest handling losses differed among the places. The lowest handling loss was recorded at Lingmethang while higher losses were recorded both at Yongkola and Tsenzebi. The mean field drying and threshing losses from these three locations were 5.0 % and 4.4 %, respectively with a total handling loss of 9.4 %. In Lingmethang, change in weight during drying and storage was minimal, while weight loss during de-husking was high and significantly different from drying and storage.

Wastage of food through post-harvest losses not just translates into human hunger but also results in lesser revenue generation for the growers (FAO, 2013, 2020). Overall, the post-harvest handling loss of quinoa in Mongar district is not alarming currently but care should be taken to minimize further losses to help achieve food security in the country. It is recommended to train farmers and relevant stakeholders on the post-harvest management of quinoa in the country to reduce the losses during post-harvest handling and operations of quinoa. It is also recommended to carry out similar studies in other quinoa-growing areas of the country to further validate the findings of this research.

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Comparative Study on Physio-chemical and Organoleptic Attributes of Apple Wine Fermented from Local and Imported Yeasts

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ABSTRACT

With home micro-brewing on the rise, use of local indigenous yeast in the fermentation process is a prevalent practice in the country. The indigenous strain found in locally processed yeast can impart certain desirable flavours and aromas to the fermented wine. Furthermore, the use of antioxidant-rich wild herbs in local yeasts is reported to enhance the nutritional value of the wine. However, local yeasts are home-made under dubious processing conditions and cannot afford the advantage of being in a pure uncontaminated microbial form, unlike the commercial strains. This might affect the fermentative capability and compromise the end quality of the wine. Thus, this study was conducted to compare the organoleptic acceptability and physio-chemical properties (total soluble solid, alcohol by volume, transmittance value, pH and titratable acidity) of apple wine fermented by two local yeasts from Trashi Yangtse and Samtse districts in Bhutan and one commercial yeast manufactured in India. Both the local yeast strains seem to yield a wine with better appearance (clarity) and taste (sweetness) based on the sensory evaluation result. In terms of fermentative capability, the commercial strain had shorter fermentation time, but the local strains were able to yield wine with similar alcohol strength to the commercial strain. For the physio-chemical parameters, only pH and titratable acidity results were significantly different with the wine from local yeast from Samtse having a significantly higher malic acid content, while the wine from commercial yeast had lower pH. However, the results deduced from the sensory evaluation does not compliment the values obtained for Total Soluble Solute and clarity measurement. Besides, the difference in pH did not resonate with the taste perception (i.e., lowest pH wine considered sweeter). Thus, other flavour components might have a role to play in the sweet taste perception. Overall, the local strains showed promising results in terms of fermentation capability (ability to convert sugar to alcohol) and organoleptic attributes of the end wine in comparison to the commercial strain.

Keywords: *Yeast, Apple wine; Physio-chemical; Organoleptic attributes*

1. Introduction

Fermentation of cereals such as wheat, barley, maize, and rice to process into alcoholic beverages has been an age-old practice in Bhutan. Some of these alcoholic beverages are *Chankey* – fermented cooked rice, *Bangchang* and *Singchang* - local beer, and *Ara*- distilled liquor Lhendup (2008). In the last few years, the National Post Harvest Center has observed a growing number of

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entrepreneurs interested in availing training and technical guidance on alcoholic fermentation from fruit juice particularly alcoholic beverages made from apple, peach, plum and apricot. Currently, the Army Welfare Project Limited, a state-owned enterprise, and Bhutan Brewery Private Limited (a unit of Tashi Group of Companies) are the two major producers and distributors of alcoholic beverages in the country.

Despite the availability of commercial alcoholic beverages in the country, home brewing of traditional beverages is still prevalent and preferred in many parts of the country (Dorji, 2012). The increasing number of people availing training on processing alcohol from surplus fruits and cereals for self-consumption is a testament to the popularity of the home microbrewery. Homebrewing of fermented liquor in the country is usually done with starter culture from the previous fermented batch or with a circular disc of local yeast starter. These yeast starter discs are processed locally in certain regions of the country. The locally cultured yeasts prevalently known as *pham* or *phaw* in the local dialect are reported to be integral to the quality of fermented beverages in Bhutan (Namgyel, 2009; Yudon, 2015).

The *Pham or Phaw* is usually made from cereals powder and grits (maize and wheat), rice husks, and dried wild herbs. The mix is inoculated with a mother starter culture by adding yeast made from a previous batch. The dried mixture is hydrated with water and kneaded into a dough; the dough is then shaped by hand into a flattened circular disc. The circular disc is either strung from a string or left on shelves above traditional mud oven to provide a warm environment for the culture to thrive and multiply. This is essentially the incubation phase followed by air drying phase. During the incubation phase, the nutrient-rich disc is exposed to the probable microflora of wild yeast (indigenous non-Saccharomyces yeast) present in the air, this exposure could lead to the contamination of the disc. Due to lack of microbiological analysis to identify the strain of yeast; at this juncture, the *pham* can contain either wild yeast or Saccharomyces or both. Due to this uncertainty, the yeast in the *pham* will be referred to as indigenous yeast strain in this paper.

Wild herbs are considered one of the most vital ingredients in the *pham.* Often wild herbs are collected from the forest, dried and added to the wheat and maize powder. These wild herbs are locally known as *bainang yangrim, chong yang rim* (Yudon, 2015) and *khagai numba/ru yangrim* (Bhutan Cultural Atlas, 2020). *Bainang yangrim* is identified as *Hedyotis pinifolia, chong yangrim* as *Buddleja asiatica* (Grierson & Long, 1983, 1984) and *khagai numba/ru yangrim* as *Hedyotis scandens* (Thinley, 2010*)*. Wangchuk, Yeshi, and Jamphel (2017) have also documented the use of *Buddleja bhutanica* (*Scrophulariaceae*) leaves also known in traditional Tibetan medicine (*Sowa Rigpa*) as *Chang-rtsi* in yeast making. For this study, *Hedyotis scandens* was added to the local yeast from Trash Yangtse (Jigme, personal communication, January 5, 2020) and *Buddleja asiatica* in the local yeast from Samtse (K. Norbu, personal communication, January, 6, 2020). As per Arjun, Verma, and Prasad (2014) extracts from both the plants are used in a similar manner as growth supplements for yeast prepared for fermenting *judima:* a local alcoholic beverage prepared by the *Dimasa* tribes of Assam in India. As per the study, the plant extracts were found to be a good source of antioxidants namely polyphenols, alkaloid and flavonoid.

Yeast is integral to the fermentation of alcoholic beverages because of its ability to convert sugar into alcohol (ethanol), carbon dioxide, and minor metabolites (Boulton, Singleton, Bisson, & Kunkee, 1999). These minor volatile metabolites are derived from the fruit as well as from the metabolic activity of the yeast. The yeast-derived metabolites are mainly organic acids, esters, alcohols, carbonyl compounds and fatty acids. These metabolites are sensorially significant and play an important role in defining the flavour and aroma of the wine (Dubourdieu, Tominaga, Masneuf, des Gachons, & Murat, 2006). Indigenous yeast contains a diverse group of yeast strain and these diverse microflora produces a plethora of secondary metabolites which makes up a complex bouquet of flavour and aroma (Lambrechts & Pretorius, 2000). The diversity of the microflora is dependent on the geographical location and climatic conditions, so wine fermented with indigenous yeast often imparts a unique flavour and aroma attributes characteristic of a particular region (Eder, María, Reynoso, Lauret, & Rosa, 2017). The floral flavour and an aroma characteristic of the wine produced in Muscat or the volatile fruity wine of the Sauvignon are all examples of how the diverse indigenous microflora native to the afore-mentioned regions impart a unique taste and aroma profile to the wine (Dubourdieu et al., 2006).

Despite the desirable complexity in the flavour and aroma produced by the indigenous yeasts, it is also imperative to understand that indigenous yeast could be unpredictable resulting in the brewer having lesser control over the fermentation. This unpredictability of the indigenous yeast will lead to inconsistent quality of the final product. Moreover, unhygienic conditions during the local yeast making process and unprotected condition of storage lead to the presence of contaminants such as dirt, dust, insect and hair particles in the *pham* proving detrimental to the safety and quality of the end fermented product. Thus, starter culture of selected commercial strains of Saccharomyces is used to ensure consistency and predictability in the wine quality. *Saccharomyces cerevisiae* is the most commonly used yeast strains commercially available for alcoholic fermentation (Andorrà et al., 2019). This particular strain is preferred for brewing because of its ability to tolerate high alcohol content, anaerobic condition and ability to convert sugar into alcohol. However, widespread commercial use of this strain often leads to a monotonous repetition of flavour and aroma, stripping off the uniqueness that is desirable in wine. Winemakers still argue that commercial strain does not add to or enhance the regional character of the wine and some still prefer spontaneous fermentation with indigenous yeast over the use of commercial strain (Rainieri & Pretorius, 2000).

Physiochemical parameters such as TSS (Total Soluble Solids), pH (hydrogen ion concentration), titratable acidity and ABV% (Alcohol by Volume %) can give important insights into the organoleptic attributes and fermentative capability of the yeast. These parameters may differ with different yeast strains because of the specific metabolic pathway unique to the individual strain

(Sharma, Singh, & Sawant, 2012). Organoleptic acceptability can be assessed through sensory evaluation of the end product by a panellist.

TSS gives a measure of sugar content and thus can be used as an indicator for sweet taste in wine. The alcohol content will contribute to the mouthfeel and after taste of the wine. Acid content and strength also have an important bearing on the flavour perception in wine (Chidi, Bauer, & Rossouw, 2018). The acidity in wine is due to the presence of dissociated and un-dissociated organic acid in fruit. Malic acid is the predominant organic acid found in apple followed by other organic acids such as citric, quinic glycolic, succinic, lactic, galacturonic and citramalic (Dharmadhikari, 1996). Titratable acidity and pH are the two important variables that express acidity in the wine. The pH scale is used to measure the alkalinity or acidity of a solution and is calculated as the negative of the base-ten logarithm of the molar concentration of hydrogen ion released by the dissociation of organic acids. It is measured on a 1-14 scale with 1 being highly acidic, 7 neutral and 14 being highly alkaline. The scale is logarithmic, meaning there is a difference of 10 times in 1 pH unit. On the other hand, titratable acidity measures the potential hydrogen ion than can be dissociated. It is measured by titration with a strong base (usually sodium hydroxide) to a neutral endpoint pH of 7.00 and is expressed as mg of organic acid per litre of sample. Thus, pH measures the strength of the acid (hydrogen ion concentration) and titratable acidity measures the concentration of acid (Comuzzo & Battistutta, 2019).

With the prevalent use of *pham* to ferment alcohol in the country, it is important to study the fermentative capability of the local *pham* and the end quality of the fermented alcohol in comparison to that of commercial strains of *Saccharomyces cerevisiae.*

Thus, the objectives of this study were:

a) to compare the physio-chemical properties of wine fermented by two different local yeast made in Bhutan and a commercial yeast from India.
b) to study if the yeast has attributes that contribute to the difference in sensory acceptability of the wines.

2. Materials and Methods

The experiment was conducted in the food analysis laboratory at the National Post Harvest Center (NPHC) of the Department of Agriculture. Apples stored in the cold store at 4 ^{0}C were washed in 0.1 % water solution of potassium metabisulphite. All the utensils and equipment used were rinsed with potassium metabisulphite solution to reduce the possibility of contamination by other microbes. The juice extraction was done in two stages: first in the juice pulper to extract juice from raw apples. Crushed apples and juice were obtained from the pulper. Further, the crushed apple flesh was squeezed in the hydraulic juice press to extract the remaining juice. The TSS of the juice was adjusted to 22 ^{o}Brix by addition of sugar. Pectinase and amylase were added at 0.03 % to clarify the juice. The material preparation method was adapted from Kanwar and Keshani (2016). The local yeast from Trashi Yangtse (LYT) was obtained from a shop in Trashi Yangtse, local

Yeast from Samtse (LYS) from a shop in Baangdey, Paro, and the Indian commercial yeast (CYI) from an Indian supplier. Both the LYT and LYS were crushed and sieved to remove unwanted dirt and dust particles. The CYI was in powdered form and was used directly. The yeasts were inoculated at 0.3 % in the juice. Potassium metabisulphite at 0.01 % was added to prevent contamination by bacteria and moulds.

After one week, the wine was racked to separate sediments (lees) and flocculation from co-precipitated tannins and protein to prevent off flavour development (Jackson, 2008). Each treatment was replicated three times and each replication weighed 10 litres. The TSS and pH were measured each day until the values for the measured parameters remained unchanged for 48 hours. This marked the end of fermentation whereby there is no further conversion of sugar to ethanol (Nout, 2014). The TSS for the samples were measured by a digital handheld refractometer (Atago PAL 3, 0-93% range with temperature compensation) and the TSS is expressed as degree Brix. The refractometer was calibrated before use with distilled water as blank to give a measurement of zero-degree Brix. The pH was measured by a handheld digital pH meter (EcoTestr pH1, Range – 0.00- 14.00). Since the predominant organic acid in apple is malic acid, the total titratable acidity was measured and expressed in terms of malic acid with titration done against 0.1N NaOH using phenolphthalein as an indicator (AOAC, n.d.).

The alcohol percentage was measured in ABV%, i.e., the volume of ethanol in litres per 100 litres of wine measured at 20 °C and expressed as % vol. The OIV-MA-AS312-01A method was used to obtain the distillate from the sample and an alcoholmeter was used to obtain the reading. The actual % ABV was obtained from the standard alcohol temperature correction table. An advanced microprocessor UV-VIS Single Beam Spectrophotometer (LI-295) was used to measure the transmittance value of the fermented wine at 660 nm with distilled water as blank. The blank was used to calibrate the spectrophotometer to give a value of 100 % transmittance or 0 % absorbance (Berutu, Fahrurrozi, & Meryandini, 2017).

Descriptive test and sensory evaluation were done on taste, colour, aroma, clarity, mouthfeel, and alcohol strength and after taste on the fermented wines. The 5-point hedonic rating scale (Table 1) adapted and modified from Jones, Peryam, and Thurstone (1955) was used to evaluate and score the samples.

Table 1. Five-Point Hedonic Scale.

Hedonic rating	Score
Like extremely (LE)	5
Like moderately (LM)	4
Neither like nor dislike (N)	3
Dislike moderately (DM)	2
Dislike extremely (DE)	1

A total of 36 panelists comprising 21 females and 15 males performed the sensory evaluation and the descriptive test. For the descriptive test, subjective descriptors such as for taste (sweet, neither sweet nor sour or sour), colour (pale yellow, darkish yellow or brown), aroma (weak or strong), clarity (clear to hazy), mouth feel (thin to full), alcohol strength (low to high) and after taste (mild to harsh) were used.

One-way Analysis of Variance (ANOVA) and cross-tabulation were done to study if the yeast type had a significant effect on the sensory evaluation scores and physio-chemical parameter of the fermented wine. The analysis was carried out using the Statistical Package for Social Science software (SPSS). P values ≤ 0.05 were considered significant in all the analyses. The mode of the scores for sensory evaluation was taken as the representative score for each attribute.

3. Results and Discussion

3.1. Total soluble solids

Total soluble solids (TSS) is the measure of the total amount of solids soluble in a sample (Garner, Crisosto, Wiley, & Crisosto, 2008); thus TSS was used to express the total amount of sugar in a solution expressed as degree Brix. The concentration of TSS was determined by passing a light through the sample and measuring the refractive indices (a measure of the bending of light rays in a medium) with a refractometer. During the fermentation process, the TSS value is expected to drop as the yeast converts sugar into alcohol and carbon dioxide. Figure 1 shows a sharp drop in TSS at the beginning of the fermentation until the 10th day after which the TSS dropped at a slower pace, and after the 16th day, there was not much change in the TSS.

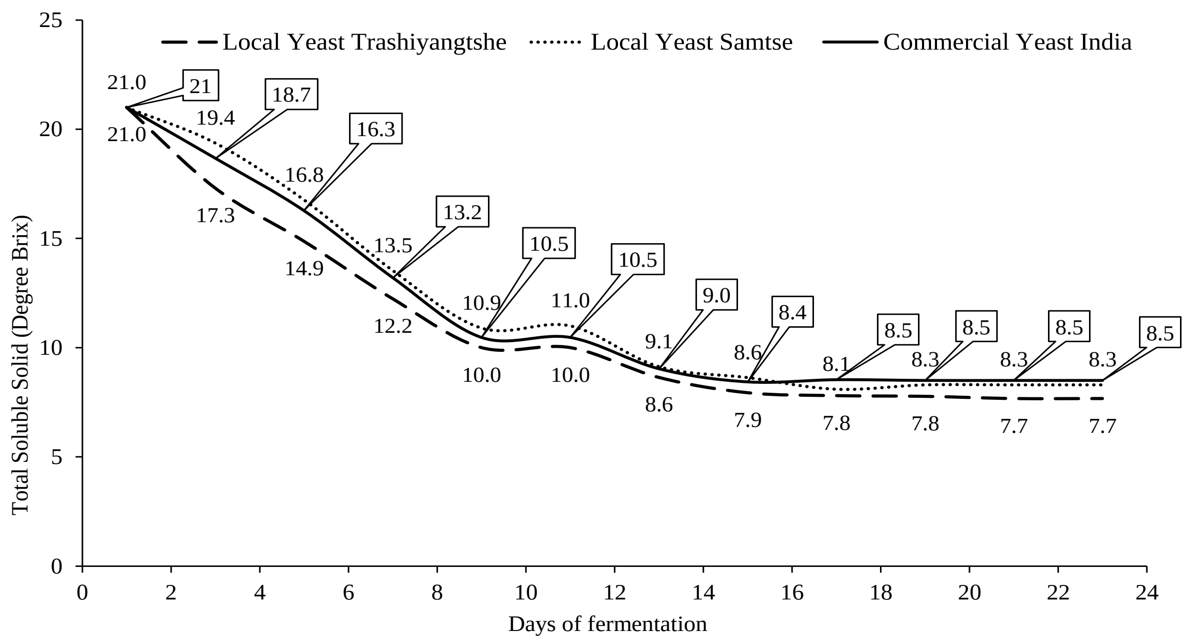

Figure 1. Average TSS in wine fermented by the three yeast strains over 23 days.

With all three yeast strains, there is a decline in the TSS reading with time, this decline is more pronounced at the beginning of the fermentation as is evident in Figure 1. This stage is called the primary fermentation stage. This decline in TSS is due to the alcoholic fermentation where the yeast feeds on the sugar and releases ethanol, carbon dioxide, energy and other minor metabolites. The amount of sugar present in the juice thus decreases and the ethanol content increases with time. As fermentation continues, increase in alcohol content and the decrease in pH suppresses the activity of yeast to convert sugar to alcohol and carbon dioxide. As a result, the decline in TSS is at a slower rate and eventually stops and this signals the end of the fermentation period (Nout, 2014).

The CYI have the shortest fermentation duration of 17 days, followed by 19 days for LYS and 21 days for LYT. All the three yeasts exhibit a similar fermentation pattern as is indicated in Table 2, where the TSS of these wines from all the threes yeasts is not significantly different.

Table 2. One-way ANOVA and post hoc Tuckey test (values expressed as Mean ± Standard error).

	Total Soluble Solute (ºBrix)	**Alcohol by Volume (ABV%)**	**Transmittance value**	**Titratable acidity (malic acid gm/L)**	**pH**
Local Yeast Samtse	7.66±0.71	11±0.57	90.76±0.95	3.7±0.00[a]	3.8±0.00[b]
Local yeast Trashi Yangtse	8.30±1.00	10.66±0.88	89.50±2.79	3.2±0.01[b]	3.7±0.03[b]
Commercial Yeast India	8.50±0.30	10.00±0	93.5±0.72	3.1±0.00[b]	3.9±0.00[a]

Means within a column with different superscripts differ significantly ($P \leq 0.05$)

3.2. pH and titratable acidity

The Post Hoc Tuckey test revealed a statistically significant difference in the measurement for titratable acid content ($F(2,6) = 7.341$, $P = 0.024$). The test showed that the wine fermented from LYS have significantly higher titratable malic acid content as compared to wine fermented from LYT and CYI (Table 1).

In terms of pH, the wine from CYI had significantly higher pH ($F (2, 6) = 13.00$, $P = 0.007$) as compared to the other two wines meaning the wine from LYS and LYT are comparatively more acidic than the CYI wine. A difference of 0.1 (=pH CYI – pH LYT) and 0.2 (=pH CYI – pH LYS) As per Murrell (2011) a difference of 0.1 pH and 0.2 pH unit translates to a solution being 1.3 and 1.6 times more acidic; thus, the wine from LYS and LYT are 1.3 and 1.6 times more acidic than CYI.

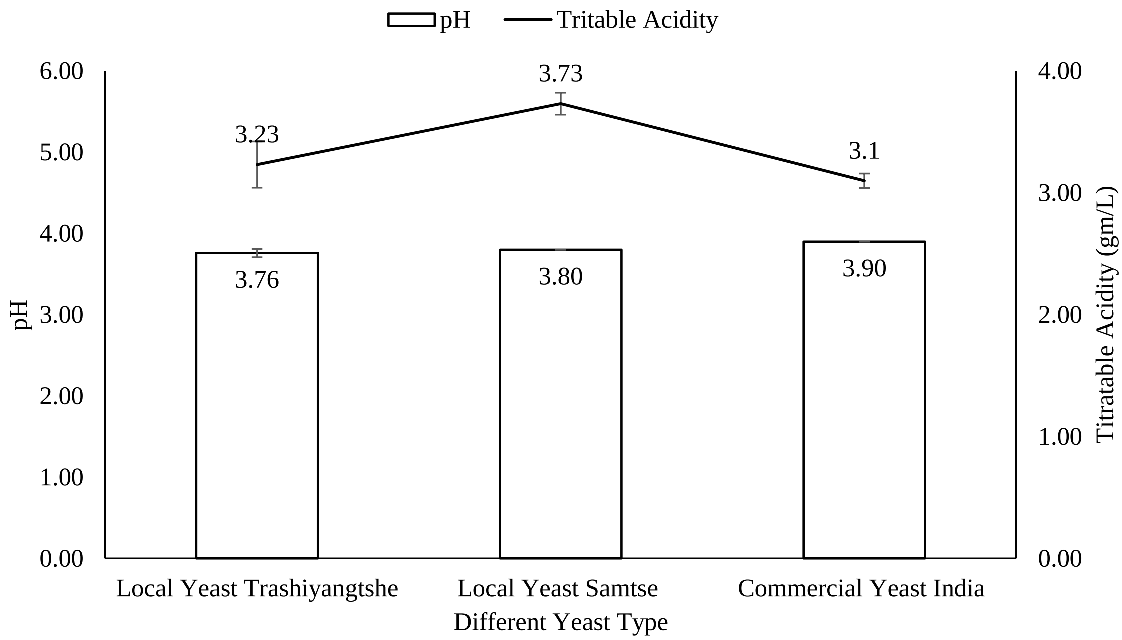

Figure 2. Average titratable acidity (gm malic acid per 100 ml of sample) and hydrogen ion concentration (pH) of the wine fermented by LYT, LYS and CYI.

3.3. Alcohol % by volume (% ABV)

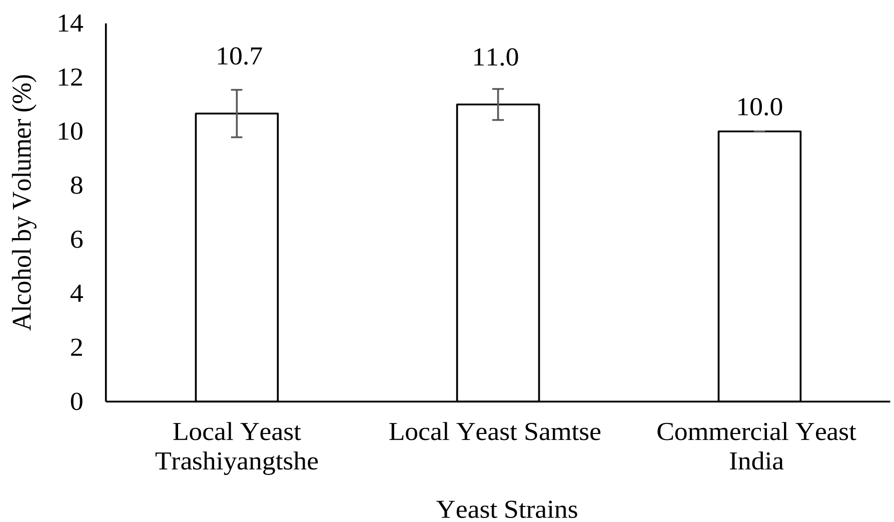

Figure 3. Average Alcohol by Volume (%) in wine fermented by LYT, LYS and CYI.

The highest average ABV% was 11 % found in the wine from LYS, followed by 10.7 % for the wine from LYT and 10 % for the CYI. However, this difference is not statistically significant as is evident from Table 2.

3.4. Clarity expressed as transmittance value

Transmittance value (expressed as percent transmittance) measured in spectrophotometer is the amount of light that passes through the sample (Garner et al., 2008) hence this value was used as a measure of clarity. The wine from CYI had the highest transmittance % followed by the wine from LYT and LYS. However, this difference is not statistically significant (Table 2).

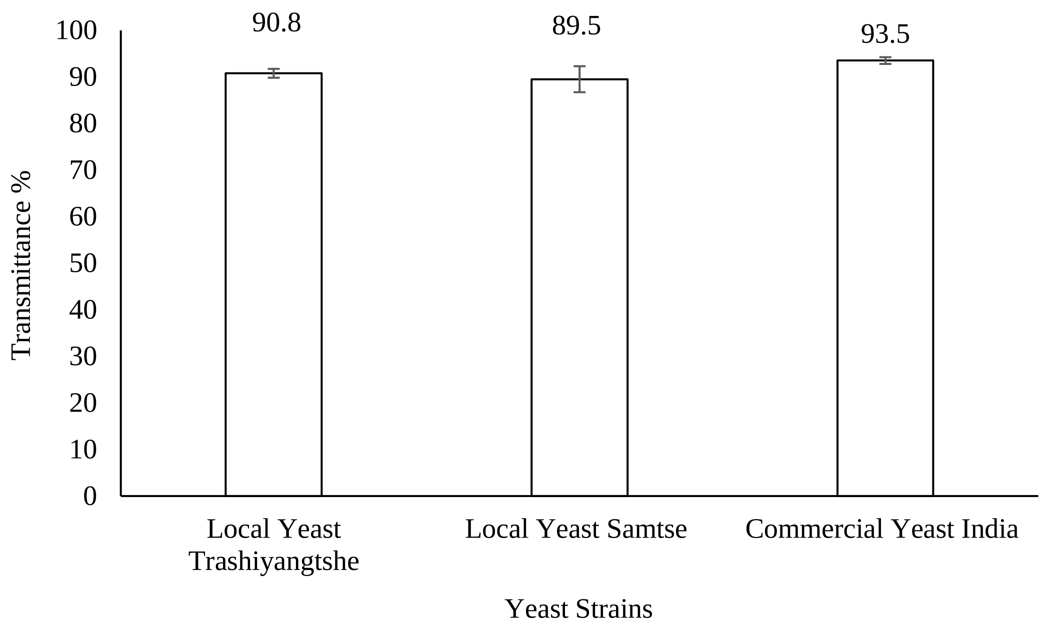

Figure 4. Transmittance% in wine fermented by LYT, LYS and CYI.

3.5. Sensory evaluation

A crosstab was done on SPSS to determine the mode to establish the most frequently occurring score as is given in Table 3. Since the mode is the most common score that majority of the panellist gave for each wine, it was used as the final scores for the attributes of different wine as presented in Figure 5. The highest percentage of panellist is highlighted in Table 3 and the score corresponding to the highest percentage is taken as the final score.

Table 3. Cross of the percentage of panellist and their hedonic scores.

	Local Yeast Trashi Yangtse					Local Yeast Samtse					Commercial Yeast India				
	DE	DM	N	LM	LE	DE	DM	N	LM	LE	DE	DM	N	LM	LE
Taste	9	15	15	29	32	6	12	24	53	6	3	9	21	50	18
Aroma	6	13	16	42	23	6	12	26	41	15	6	12	30	39	12
Clarity	3	3	28	44	22	3	9	29	26	32	3	0	39	48	9
Mouthfeel	3	16	19	38	25	3	18	21	56	3	3	9	27	48	12
Alcohol strength	3	16	16	59	6	3	6	21	59	12	3	6	18	70	3
After taste	3	13	19	45	19	6	6	27	39	21	3	9	30	42	15

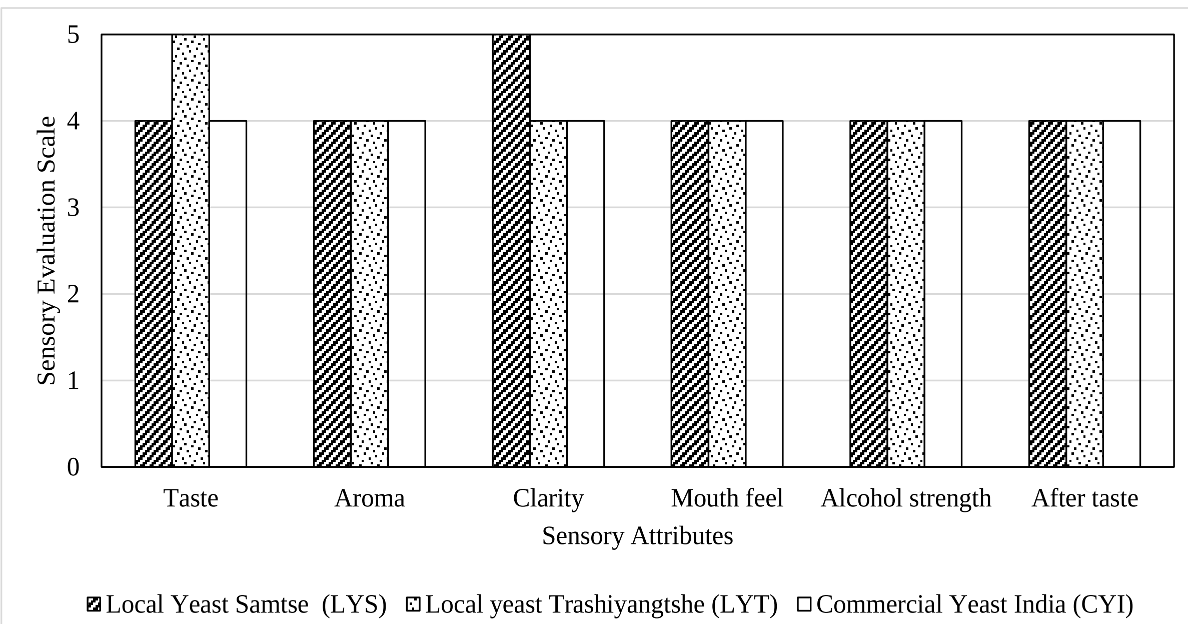

Figure 5. Horizontal column graph of the sensory evaluation score.

Majority of the panellists scored “liked moderately” in all the attributes for all the three wines except for clarity and taste (Figure 5). LYS wine had the highest percentile i.e., 32 % of the panellist gave “extremely liked” score to the clarity attribute. Majority of the panellist i.e., 78 %

described the LYS wine to be clear. LYT wine had the same result with 32 % of panellist giving "extremely liked" score to the taste attribute. The descriptor sweet for taste attribute was used by the majority of the panellist i.e., 53 %.

3.6. Discussion

Based on the result of the one-way ANOVA and Tukey's test for post hoc analysis (Table 2), there is no significant difference in the TSS, ABV% and Transmittance %. In terms of fermentative capability, the CYI had a shorter fermentation time followed by the LYS and LYT. However, no significant difference was found in the final ABV%. Commercial yeast strains are well defined pure yeast culture selected because of their superior fermentative capacity and hence often are considered better in terms of fermentative capability (Steensels et al., 2012). In this study though the CYI had shorter fermentation time, the local strain produced wine with similar alcohol strength to the CYI. Thus, in terms of the ability to convert sugar into alcohol, the performance of all the three strains is at par with each other.

However, there is a statistically significant difference in the measurement for titratable acid content with the wine from LYS having higher titratable malic acid content as compared to wine fermented from LYT and CYI. This result agrees with the interpretation of pH measurement whereby the LYS had the highest acidity and CYI the lowest. The LYT wine was extremely liked for its sweet taste by the majority of the panellists; however, there is no significant difference in the TSS amongst all the three wines. Therefore, it can be speculated that the TSS in this case did not have any bearing on the perception of sweet taste. However, the pH value records a significantly lower value for LYT which can be interpreted as the wine being sourer than the other two. The TSS and pH measurements contradict the perceived taste of sweetness, and thus there might be factors other than TSS and acidity attributes responsible for the perception of the sweetness in the wine. The use of the wild herbs in the local yeast might be a contributing factor towards the perceived sweet taste in the wines processed from local yeast. However, as there is no prior research done on sensorially significant compounds in the aforementioned wild herbs it is difficult to establish their impact on the final sensory attribute of the wine.

Lambrechts and Pretorius (2000) have reported the effect of different yeast strains on production and accumulation of succinic acid and phenolic compound in wine which can impart a bitter and astringent taste. It can be speculated that the presence of succinic acid and phenolic compound in the other two wines had an impact on the panellists perceiving the other two wine as less sweet than the LYT. A similar finding was also reported by Bandić et al. (2018). Furthermore, Marchal, Marullo, Moine, and Dubourdieu (2011) reported on the possible contribution of yeast autolysis phenomenon and subsequent production of Hsp12p strain of protein on the sweetness on wine. Since the study of these factors was beyond the scope of this work, one can only speculate on the plausible causes. The result of the transmittance value gave no significant difference amongst the three wines, yet the panellists seemed to prefer the clarity of the LYS. Since the sensory evaluation is subjective in nature the reason why the panellists preferred wine from LYS in terms of clarity is inconclusive.

4. Conclusion

The study found that the local yeast strains fermented apple wine with better sensory acceptance especially in terms of taste and clarity attributes. This finding is in agreement with the previous studies that have highlighted the impact of using indigenous strains on the development of desirable flavour and aroma in the wine. In terms of fermentation capability, though the local strains take longer time to reach the end fermentation point, the alcohol strength of the end wine is not significantly different from the commercial strain. The fermentation capability to convert sugar to alcohol is similar in the commercial and local strains despite the commercial strain having the advantage of being adaptively selected and cultured in pure form. It is possible to enhance the fermentation capability if the local strains are to be cultured in the pure form under controlled conditions. The literature reviewed also reveal that the use of wild herbs in the local yeast enhances the antioxidant content of the wine. Thus, it can be concluded that local yeast strains have comparable fermentative capability with the potential to possibly surpass commercial strains if it is cultured in pure form like commercial strains. In terms of organoleptic attributes, the local strains yield a wine with better acceptability and with an enhanced nutritional value from the addition of the wild herbs.

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Assessment of Nursery Methods and Manures for Cultivation of Chirayita (*Swertia chirayita* Buch-Ham.) in Lauri Gewog, Bhutan

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ABSTRACT

Chirayita (Swertia chirayita) is a medicinal plant and is native to the temperate Himalayas. It is widely used in traditional and modern medicines with an increased global demand, leading to unsustainable harvesting, causing the decline, and critically endangering the species. Hence, we hypothesized that chirayita could be domesticated and tested this hypothesis at Lauri Gewog in southeastern Bhutan. Four nursery methods and four organic manure treatments were tested using Completely Randomized Design with three replicates. The average seedling density across the four treatments at mid altitude was 2356 seedlings/m^2, and that of high altitude was 1202 seedlings/m^2. The seedling density at mid altitude was 96% higher compared to that of high altitude; however, there were significant differences between the treatments only in high altitude ($P = 0.003$) and not in mid altitude. The methods were non-significant at mid altitude, mainly due to the large variability of data. Interestingly, the manure treatments were not significant compared to the control implying that the chirayita could be non-responsive to the application of manures in the study conditions. Fresh weight of chirayita in the control plot was 14, 19 and 29% higher than 'compost', 'FYM' and 'compost+FYM (1:1)' treatment plots. Further, this research provides an alternative low-cost nursery technique for adoption by chirayita farmers while also providing information for policymakers and environmental conservationists in developing strategies for conservation of a critically endangered species like chirayita.

Keywords: *Swertia chirayita; Nursery methods; Manure application; Seedling density; Conservation; Domestication*

1. Introduction

Chirayita *(Swertia chirayita* Buch-Ham.) is a medicinal plant indigenous to the temperate region of the Himalayas (Bhargava, Rao, Bhargava, & Shukla, 2009; Naveen, Suresh, Chhavi, & Ritu, 2017; Scartezzini & Speroni, 2000). It is found at altitudes ranging from 1200 to 3000 meters above sea level (masl) in Bhutan, India and Nepal (Aleem & Kabir, 2018; Balaraju, Agastian, & Ignacimuthu, 2009; Bhargava et al., 2009; Khanal, Shakya, Nepal, & Pant, 2014; Shukla, Dhakal,

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Uniyal, Paul, & Sahoo, 2017). It is popularly known as ‘chirayita’. The use of this species is reported in many traditional medicine systems viz. Ayurveda, Bhutanese *gSo-ba Rig-pa*, Unani, Siddha, Tibetan, Yunami, British and American pharmacopoeias (DoF, 2008; Joshi & Dhawan, 2005; Negi, Singh, & Rawat, 2011; Pradhan & Badola, 2015a; Sabita, Lal, Kumar, & Amrita, 2019; Saha & Das, 2010; Scartezzini & Speroni, 2000). It is used to treat a wide range of ailments (Brahmachari et al., 2004; Kumar & Staden, 2016; Pradhan & Badola, 2015a). It is valued for its active compounds such as amarogentin, amaroswerin, gentianine, swertinin, swerchirin, mangiferin, lignan, triterpenoid, decussatin and isobellidifolin (Bhargava et al., 2009; Brahmachari et al., 2004; Joshi, 2008; Joshi & Dhawan, 2005; Scartezzini & Speroni, 2000; Tabassum, Mahmood, Hanif, Hina, & Uzair, 2012). It is reported to possess numerous medicinal properties such as antipyretic (Bhargava et al., 2009), anthelmintic (Iqbal, Lateef, Khan, Jabbar, & Akhtar, 2006), anti-inflammatory, anti-diabetic (Karan, Vasisht, & Handa, 1999), antibiotic (Roy et al., 2015) and anti-carcinogenic (Saha & Das, 2010).

The traditional medicine systems use the whole plant (Bhargava et al., 2009; Tabassum et al., 2012), wherein the entire plant, including its roots is uprooted before seed setting, depriving the species of its natural regeneration potential (Aleem & Kabir, 2018). Further, the natural regeneration of chirayita is also affected due to poor seed germination and low viability (Chakraborty, Mukherjee, & Baskey, 2016; Chaudhuri, Pal, & Jha, 2007, 2008, 2009). Due to over-exploitation, unsustainable harvesting practices and habitat loss, the International Union for Conservation of Nature (IUCN) listed the chirayita as one of the critically endangered plant species (Joshi & Dhawan, 2005; Kumar & Staden, 2016). This was why India banned the trade of wild *Swertia chirayita* since 2004 (Cunningham, Brinckmann, Schippmann, & Pyakurel, 2018). On the contrary, the regional and international demand for chirayita is on the rise every year. According to Chaudhuri et al. (2007), the annual demand for chirayita in the Indian subcontinent alone is 400 tons, with an annual growth rate of 10 %. Similarly, Cunningham et al. (2018) estimated an annual export volume of 489.70 to 698.90 tons from Bhutan, India and Nepal of which Bhutan’s share constituted about 19 %. The export trend of chirayita from Bhutan (2009 to 2019) (Figure) demonstrates the importance of chirayita for Bhutanese farmers. The majority of the market demand is still met from the wild collection, and a limited share comes from cultivated sources as it is generally not cultivated due to the lack of standard cultivation package of practices (Badola & Pradhan, 2011). However, the past evidence shows that attempts were made to encourage and promote the cultivation of the chirayita in Bhutan (DoFPS, 2012). In recent years, there is a rising interest of pharmaceutical companies in cultivated chirayita for authenticity and sustainability reasons (Badola & Pradhan, 2011). Further, there is a growing concern among the consumers of pharmaceutical products on whether a product has sustainable source. This impetus from market forces provides massive scope for domesticating medicinal plants like chirayita for commercial cultivation. However, there is a lack of understanding of how this species can be cultivated and this study attempts to investigate its domestication potentials under Bhutanese conditions.

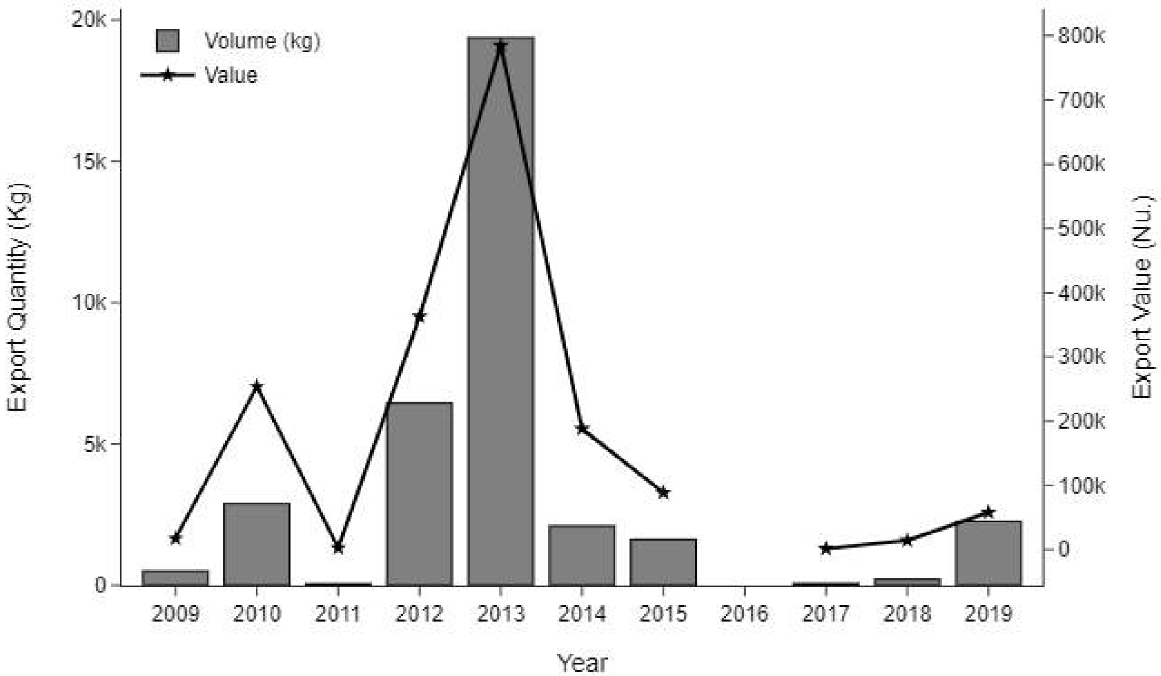

Figure 1. Chirayita export volume (kg) and value (Nu.) from Bhutan (2009-2019), Source – Bhutan Trade Statistics (2009 – 2019).

The people of Lauri Gewog (block) under Samdrup Jongkhar district in Bhutan depend on Non-Wood Forest Products (NWFPs) for cash income, employment, and livelihood. Their dependency and livelihood are at stake due to the depletion of wild chirayita in the forest coupled with the decrease in the market price for wild chirayita in recent years (DoF, 2008). This is a serious concern to the farmers, local leaders and conservationists in Bhutan. The farmers attribute the decline of chirayita plants to the government policy on banning the 'slash and burn' farming system. They believe that the 'slash and burn system' would stimulate the natural regeneration of chirayita. This is further compounded by the indiscriminate and unsustainable harvesting of chirayita from the wild. The resource assessment carried out by the Department of Forests further supports the depletion of wild chirayita resources (DoF, 2008).

Hence, there is an urgent need to domesticate and cultivate chirayita on-farm for a sustainable supply chain and its conservation (Badola & Pradhan, 2011; Jäger & Staden, 2000; Lubbe & Verpoorte, 2011; Phondani et al., 2016; Shukla et al., 2017; Wiersum, Dold, Husselman, & Cocks, 2007). While there were studies that recommended different propagation techniques, including in-vitro propagation (Balaraju et al., 2009; Chaudhuri et al., 2007, 2008, 2009; Pradhan & Badola, 2008, 2011, 2012), such technologies are too expensive and remained inaccessible to the farmers. There is a need to generate low-cost cultivation techniques, including nursery methods and cultivation practices easily accessible and adoptable by farmers. Therefore, the present study attempted to domesticate *Swertia chirayita* with the following specific objectives: (i) to evaluate low-cost nursery methods and (ii) to evaluate yield and yield parameters with different manure treatments.

2. Materials and Methods

2.1. Study Site

The study was conducted in two villages of Tshothang and Dungmanma in Lauri Gewog under Samdrup Jongkhar district in the southeastern part of Bhutan between 2015 to 2017 (Figure). The gewog shares its border with the Arunachal Pradesh state of India in the East, Merak Gewog of Trashigang Dzongkhag in the north and Serthig Gewog in the south. The gewog has a total area of 273.4 sq. km. It lies between the longitude 91˚47’26 E’’ to 92˚ 02’28 E’’, and latitude between 27˚ 04’94 N’’ to 27˚ 14’37 N’’ with altitudes ranging from 1800 - 2600 masl. Maize, paddy, upland paddy, millets, buckwheat, and some vegetables are cultivated as the primary food crops (MoAF, 2020). The people earn cash income mainly from the sales of NWFPs particularly, *Rubia cordifolia*, *Paris polyphylla*, *Litsea cubeba* and *Swertia chirayita*.

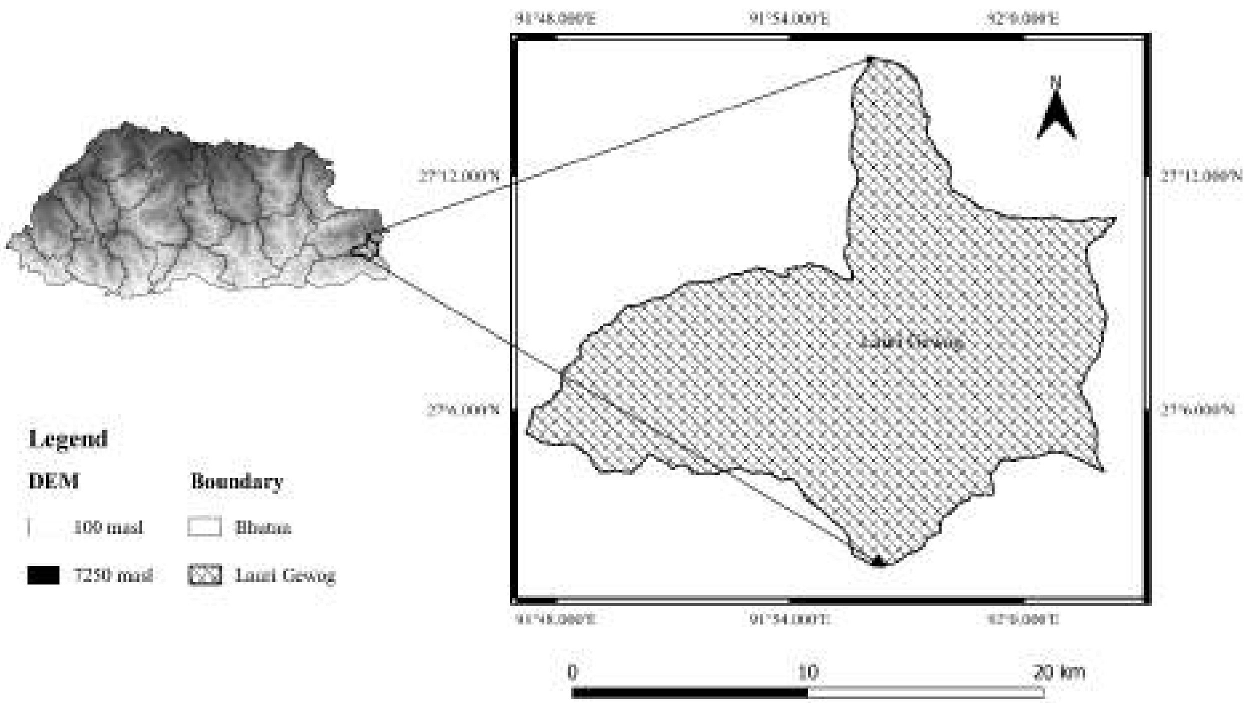

Figure 2. The study site of Lauri Gewog under Samdrup Jongkhar district.

2.2. Experiment Design

All the experiments under the present study were carried out in open field conditions following the recommendations by Badola and Pradhan (2011) for chirayita cultivation in Sikkim. The method also has reference to the findings of Pradhan and Badola (2015b) where chirayita was found to do well in the open field condition in its natural habitats. Both the nursery (detailed in section 3.2) and cultivation (with different organic manures and combinations) experiments were assessed using Complete Randomized Design (CRD) with three replications as the field was fairly homogeneous. The farmer method was used as the control for the nursery experiment. For the cultivation experiment, four types of manure treatments; (i) farmyard manure (FYM) @ 10 t/acre, (ii) compost (based on crop residues and cattle manure) @ 10 t/acre, (iii) Compost and FYM (1:1) @ 10 t/acre and (iv) control without any organic manures were applied.

Seeds collected from the healthy wild population in November 2014 were cleaned, processed (without any seed treatment), and sown at the rate of 6 g per treatment plot. Care was taken to separate external materials, including weed seeds. The seed sowing was done in March - April 2015 (Mukherjee, 2013; Shukla et al., 2017). Manual weeding and routine irrigation were provided to growing seedlings as and when required.

The transplanting of chirayita seedlings in the main field was done five months after sowing when the seedlings attained a 3-5 leaf stage and when the seedlings were strong enough to withstand transplanting shock. The chirayita seedlings (32 numbers) were transplanted on raised beds (1m x 2 m), maintaining row to row and plant to plant distance of 15 cm. The entire trial plots were fenced to ensure protection for the growing plants.

2.3. Nursery Methods

There were four nursery raising methods assessed in the study. The control used was the farmer method. The following section is devoted to providing details of each method for repeatability/reproducibility.

Burning method

This method mimics the slash and burn practice usually practiced by farmers who believe that chirayita grows better in burnt areas. The soil was dug or ploughed three times to prepare raised beds of 1m x 2m dimensions. Stones and large clods were removed and soil was leveled. The beds were then covered with dry plant mass and burnt to ashes. Thick ashes were removed after letting the soil cool overnight. Six grams of chirayita were then broadcast using a perforated plastic bottle (seed broadcaster).

Dolokha method

This method of nursery is practiced by villagers of Dolokha village in Nepal. It was introduced by the researchers after a short study visit to Nepal in 2015. However, the method is not documented. The soil was dug or ploughed five times. Stones and large clods were removed, and the soil was sieved three times using a fine sieve. Then the soil was raised to make 1m x 2m beds. Six grams of chirayita seeds were then broadcast using a perforated plastic bottle (seed broadcaster) and covered with a thin layer of pine leaves.

Farmer method

This method is used by the farmers of Lauri Gewog in raising nurseries of small seeded vegetables. Raised beds of 1m x 2m beds were prepared after digging and ploughing the soil three times. Stones and large clods were removed and soil was leveled. Six grams of chirayita seeds per bed was then broadcast with hand.

Research method

This method is commonly used by the Medicinal and Aromatic Plants Program (MAP), National Centre for Organic Agriculture (NCOA), Yusipang for domestication research. However, it is important to note that this method is nowhere documented. The soil is dug or ploughed three times. The stones and large clods were removed, and the soil sieved two times using a fine sieve. Then the soil was raised to make 1m x 2m beds in dimension and leveled. Six grams of chirayita seeds were then sown in lines at 5 mm depth, maintaining row to row distance of 30 cm and covered with a thin layer of fine soil.

2.4. Parameters studied

Chirayita seedling parameters

The data for seedling density was collected from Tshothang (high altitude) and Dungmanma (mid altitude) villages. The seedling density per unit area was obtained by randomly throwing one square foot quadrat in the treatment plots and counting the number of seedlings within the quadrat. The process was replicated three times. The values obtained were later converted into seedling density per square meter, and an average value was calculated to represent the plot.

The chirayita seedling parameters assessed were (1) plant height, (2) leaf length, and (3) leaf width. The data were collected in August 2015 from the 10 randomly selected seedlings before transplanting (at 3-5 leaf stage). The plant height was measured from the soil surface to the tip of the tallest leaf, while leaf length and width were measured from well-developed leaves.

Yield and yield parameters of chirayita

Due to extended crop gestation period to three years in the high altitude (Tshothang) experimental site and due to unavoidable logistic challenges, chirayita harvest data was recorded only from mid altitude (Dungmanma village) in October 2016. Chirayita was harvested after flowering but before the seed setting when the stem starts turning yellowish. The yield parameters assessed include (1) fresh weight, (2) plant height, (3) root length, and (4) number of shoots per plant. The plant height was measured before the harvest, while the root length and plant biomass measurement (fresh weights) were undertaken after the harvesting operations. The number of shoots per plant were recorded during harvest. Enough care was taken to exclude extraneous materials such as weeds and soil.

2.5. Data processing and analysis

The data collected were processed and analyzed using the general-purpose programming language Python version 3.8.4 (Van Rossum, 2007, June). Various python libraries were used viz. Pandas (McKinney, 2011) for data cleaning and processing, matplotlib (Hunter, 2007), Seaborn (Waskom et al., 2014) and Plotly (Sievert, 2020) for data visualization, and Numpy (Walt, Colbert, & Varoquaux, 2011), Scipy (Virtanen et al., 2020) and Statsmodels (Seabold & Perktold, 2010, June) for statistical analysis.

The means of variables under study were compared using one-way analysis of variance (ANOVA) at a P-value (alpha) of <0.05 for statistical significance. Tukey's *HSD* post hoc test was used for multiple comparisons of means (P-value = 0.05). Descriptive statistics were used to compute the mean and standard deviation of the variables assessed. All the variable means obtained from Tshothang and Dungmanma villages are presented as high altitude (2015 masl) and mid altitude (1655 masl), respectively.

3. Results and Discussion

3.1. Chirayita seedling parameters

Chirayita seedling density

The means of seedling density per square meter obtained from both the study sites are presented in Table 9. The ANOVA showed that the seedling density per meter square amongst the four nursery methods differed significantly only for high altitude and not for mid altitude. The average seedling density across the four treatments at mid altitude was 2356 seedlings/m^2, and that at high altitude was 1202 seedlings/m^2. The seedling density at mid altitude was 96% higher compared to that of high altitude. However, there were no significant differences between the treatments at mid altitude (Table 9).

The farmer method at the high altitude obtained significantly higher seedling density per square meter than the other methods except for the Dolokha method. While the seedling density in the Dolokha method significantly differed from burning method, it did not differ with the research method in high altitude. Further, the seedling density in research method and burning method did not differ significantly in high altitude. Although not significant statistically, the burning method in the mid altitude obtained higher seedling density in comparison to other methods.

Table 9. Mean seedling density at two study sites.

Nursery methods	High altitude (Number per m^2)	Mid altitude (Number per m^2)
Burning method	481^{c}	3362^{ns}
Dolokha method	1385^{ab}	1622^{ns}
Farmer method	1191^{a}	2020^{ns}
Research method	951^{bc}	2418^{ns}
P-value	0.003	0.25

Different lower-case letters in the superscript indicate statistically significant differences following the Tukey's HSD post hoc analysis at P<0.05; ns = not significant.

Plant height, leaf length and leaf width of chirayita seedlings

The mean of the seedling parameters segregated into high altitude and mid altitude are presented in Table 10. The study found that the seedling parameters amongst the four nursery methods differed significantly for mid altitude (**Error! Reference source not found.**Table 10).

All the seedling parameters in the Dolokha method were significantly different from that in the other nursery methods in the mid altitude, while other pairwise comparisons did not show any significant differences (Table 10). On the contrary, no significant difference in the seedling parameters was observed amongst the nursery methods at high altitude (Table 10). However, the burning method had the tallest seedlings compared to other nursery methods.

Table 10. Mean seedling parameters in different nursery methods at two study sites.

Nursery method	High altitude (2015 masl)			Mid altitude (1655 masl)		
	Height $(cm)^{ns}$	Leaf length $(cm)^{ns}$	Leaf width $(cm)^{ns}$	Height (cm)	Leaf length (cm)	Leaf width (cm)
Burning method	0.88 (0.25)	1.39 (0.36)	0.60 (0.08)	$0.99 (0.04)^{b}$	$1.16 (0.21)^{b}$	$0.65 (0.15)^{b}$
Dolokha method	0.75 (0.17)	0.96 (0.19)	0.49 (0.06)	$2.05 (0.47)^{a}$	$2.76 (0.77)^{a}$	$1.18 (0.08)^{a}$
Farmers method	0.84 (0.02)	1.22 (0.10)	0.53 (0.01)	$1.11 (0.25)^{b}$	$1.35 (0.43)^{b}$	$0.74 (0.09)^{b}$
Research method	0.76 (0.22)	0.93 (0.16)	0.48 (0.08)	$0.92 (0.13)^{b}$	$1.02 (0.17)^{b}$	$0.57 (0.10)^{b}$
P-value	0.817	0.145	0.32	0.004	0.007	0.003

Different letters in superscripts indicate statistically significant differences following Tukey's HSD post hoc analysis at P<0.05; ns = not significant; values in parentheses indicate standard deviations of the means.

It was found that across both sites, on average, all four nursery methods performed better in mid altitude compared to the high-altitude. The poor performance of nursery in the high altitude could have been due to low temperature affecting the seedling growth and development, resulting in shorter height, leaf length, and width. The better performance in the mid altitude could be due to the fulfillment of optimum temperature required for proper growth and development of chirayita seedling. A similar finding was reported in feasibility research of two *Lesquerella* species in

Arizona (Dierig, Adam, Mackey, Dahlquist, & Coffelt, 2006). The significant performance of the Dolokha method at the mid altitude could be possibly due to the combined effect of higher moisture retention by applied mulches and conducive conditions. Further, the performance of different nursery methods did not follow the same trend at the two altitudes, which could be attributed to variation in the micro-climatic conditions.

The findings from the current nursery trial provide an alternative and cheaper propagation method against the current backdrop of expansive and non-adoptable propagation techniques such as tissue culture and in-vivo propagation. It also indicates that the farmers of Tshothang (high altitude) can adopt either 'Dolokha' or 'burning' method, while the farmers of Dungmanma (mid altitude) are recommended to adopt either 'Dolokha or 'farmer' method for raising chirayita seedlings. The current study could not capture the effect of the 'slash and burn system' on the germination and regeneration of chirayita as opined by the farmers. However, the performance of the burning method in both sites suggests the positive effect of the system on chirayita germination and regeneration. It may be noted that the results presented in this article are based on 'one crop season' data, and thus, more similar research in the future is required to substantiate our findings.

3.2. Yield and yield parameters of cultivated chirayita

The current study showed that the yield (Figure) and yield parameters (Table 11) among the treatments were statistically insignificant. However, the average yield obtained under the current study is comparable to the findings of a study conducted in Sikkim (Badola & Pradhan, 2011). It is interesting to note that the manure treatments were not significant compared to the control in terms of all the parameters assessed, implying that chirayita may be non-responsive to the application of manures under the study conditions. Also, the fresh weight of chirayita in the control plot was 14, 19 and 29% higher than 'compost', 'FYM', and 'compost+FYM (1:1)' treatments showing the possible non-responsive nature on the yield. This could be interpreted in three ways. Firstly, possibly due to the fulfillment required soil fertility (soil could have been fertile and no additional fertility enhancement needed) for the crop. Secondly, it could be because the native plants or landrace are rather negatively affected by excess fertilizers since they are adapted to natural conditions. Thirdly, the possible non-responsive nature of chirayita to the local conditions of Lauri Gewog.

Further, the results hint that the application of manures could retard agronomic productivity. Similar findings were reported by Samarakoon (1997), where the combined application of N, P and K treatment reduced the yield of *Rauvolfia serpentina*. It is an important finding that this study has uncovered, and so there is a need to conduct a series of studies to reconfirm it. It is recommended that soil nutrients status in the experimental sites be investigated along with the agronomic parameters so as to rule out any pre-existing high soil fertility status. Non-responsiveness to manures by chirayita observed can be validated once the suspected pre-existing high soil fertility status is ruled out.

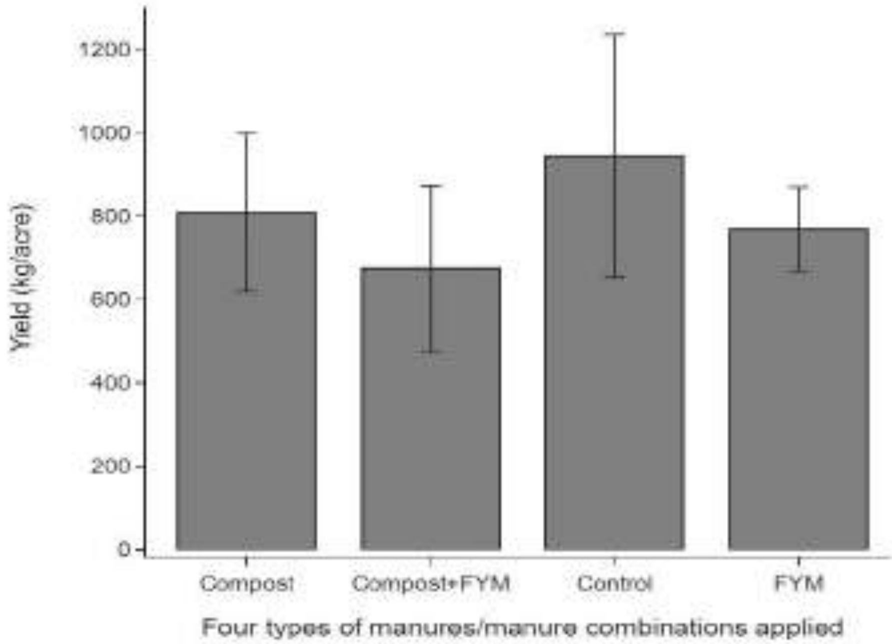

Figure 3. Fresh weight yield including roots (kg/acre) of chirayita from different manure treatments. Vertical bars indicate SE of means. There are no significant differences between treatments.

The tallest plants were observed in the treatment plot applied with 'FYM', while the longest root length and number of shoots per plant were recorded in the treatment plots that received 'compost + FYM (1:1)'. Although not significantly different, the 'control' plot produced taller plants compared to the 'compost' and 'compost + FYM (1:1)' treated plots. Similarly, root length was longer in the control plots compared to that in the 'compost' and 'FYM' treated plots.

Table 11. Mean yield parameters of plant height, root length, shoots per plant in the four manure treatments. The treatments are not significantly different for the three attributes.

Treatment	Plant height (cm)	Root length (cm)	Shoots / plant (numbers)
Compost	122.06	11.22	6.06
Compost+FYM (1:1)	112.58	12.67	6.92
Control	117.08	12.44	5.33
FYM	130.00	11.72	3.33
P-value	0.78	0.92	0.59

4. Conclusion

The findings corroborate that chirayita can be cultivated like any other crop. In the current study, the seedling density was significantly different for high altitude and not for mid altitude growing conditions. The nursery methods for seedling density did not differ significantly at mid altitude because of the large variability of data. Interestingly, the manure treatments were not significant compared to that in the control plots implying that the chirayita may be non-responsive to the application of manures in conditions similar to the study sites. The fresh weight of chirayita in the control plot was 14, 19 and 29% higher than 'compost', 'FYM' and 'compost+FYM (1:1)' treatments. Further studies are required to validate whether or not chirayita as a cultivated crop is non-responsive to manure applications.

Additionally, this research provides an alternative low-cost nursery technique for adoption by the chirayita farmers while also providing missing information for policymakers and environmental conservationists in developing strategies for conservation of chirayita - a critically endangered and vulnerable plant species.

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