Potato mini-tuber production using an Aeroponics System during winter

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ABSTRACT

This study evaluated the performance of potato mini-tuber production using aeroponics technology during winter at National Seed Centre, Paro. Three potato cultivars: Khangma Kaap (KK), Nasephey Kewa Kaap (NKK) and Yusi Kaap (YK) were evaluated in an aeroponics at two different planting times (November and December) using a split-plot design with three replications. The combined analysis, i.e., General Linear Model-Univariate was performed to test the interaction between planting times and cultivars. The interaction between planting time and cultivars showed no significant difference for any of the plant growth and yield variables. Further, planting time did not influence minituber production for the measured traits of plant height, no. of leaves, root length, number of minituber and minituber weight. However, the main effect of cultivar was highly significant (p < .01) for growth, yield and plant mortality rate. NKK yielded highest (18.39g/plant) in terms of weight followed by KK (9.22g/plant) and YK (8.30g/plant). Plants in NKK cultivar also had the maximum (8) numbers of mini-tuber per plant than KK (5) and YK (3). Maximum (39%) plants mortality was observed in YK compared to the lowest, NKK (16%). This result shows that NKK has better adaptation and is more productive under winter growing conditions than other two varieties. Thus NKK may be recommended cultivar among the three for mini-tuber production through aeroponics during winter. Since, the plants response under aeroponics conditions was cultivar dependent and not planting time dependent, further testing is recommended to select more adaptive cultivars for winter season.

Keywords: *Aeroponics; cultivars; planting time; potato mini-tubers*; *winter*

1. Introduction

Potato (*Solanum tuberosum* L) is one of the most important cash crops for farmers in Bhutan. According to the Bhutan Trade Statistic (2014), potato ranked first in terms of volume of agriculture trade and was in the top 10 export major commodities, worth Nu 688.79 million. However, there are a number of production problems in potato. Limited accessibility to high quality potato seed is a perennial problem amongst many growers in Bhutan. The annual seed potato requirement for Bhutan is approximately 10,000 ton and almost 99% of the requirement is covered through seed sources from informal system (Chettri et al 2006). In the absence of reliable sources of quality seed, many growers opt to use their own farm-saved seeds or source from the local market. Such practices contribute towards sub-optimal yields due to seed degeneration caused by viruses and others potato diseases (Roder et al 2008; Gildemacher et al 2009). To make seed potatoes available and minimize the problem of seed potato degeneration, the need of rapid multiplication has become a priority.

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In 2012, aeroponics system of potato mini-tuber production was introduced at the National Seed Centre (NSC), Paro in collaboration with the National Potato Program (NPP). It is a plant culture technique in which mechanically supported plant roots are intermittently misted with nutrient solution (Barak et al 1996). The plants are grown in air without the use of soil medium. This system has potential to produce disease free planting materials with high multiplication rate. The technique is called 3G (three generations) seed strategy (Otazu, 2010), that can lower costs and multiply seed within the three generations compared to conventional methods, which takes five to six generation. In Peru, an individual potato plant can produce over 100 mini-tubers (Otazu 2010). This contrasts with conventional methods that produce only about 5–6 tubers per plant (Hussey and Stacey, 1981; CIP 2008).

Aeroponics in Bhutan is first of its kind in South Asia (Wangchuk 2012) and the facility regarding its full potential was not explored. According to Ritter et al (2001) and Mbiyu et al (2012) potato mini-tubers can be produced twice a year using aeroponics technique. However, the facility in NSC, Paro was operated once a year (April to October) only. Therefore, this study was designed to test the feasibility of growing potato plants during winter, so that more quantity of potato mini-tubers can be produced in a year. In order to test the feasibility of winter production, two planting times (November and December) were used, because different planting time influences the performance of plants under aeroponics system (Yan et al 2000; Mateus-Rodriguez et al 2014). Similarly, three cultivars (Khangma Kaap, Nasephey Kewa Kaap and Yusikaap) were included, as many studies have found the difference in plant performance among the cultivars under aeroponics (Farran et al 2006; Otazu 2010). Therefore, the present study was conducted with the objectives to evaluate the effect of planting time and cultivars for potato mini-tuber production in winter using aeroponics technology.

2. Materials and Method

2.1. Location of the experiment

The experiment was conducted under aeroponics greenhouse (AGH) conditions in the National Seed Centre (NSC), Paro. It is located at an altitude of 2,406 meter above the sea level between 27º 22’59” N and 89º 25’12” E. The AGH dimension was 34 m long, 9 m wide and 4 m high. The duration of the study was five months (November 2015 to March 2016).

The experiments followed split-plot in a Randomized Complete Block Design (RCBD) with planting times (November and December) in main-plots and varieties (Khangma Kaap, Nasephey Kewa Kaap, and Yusi Kaap) in sub-plots with three replications. A distance of 10 cm between the plants and 15 cm between rows were maintained. Each cultivar had 180 plants and in total, there were 540 plants. The temperature inside the AGH was recorded hourly using HOBO data logger (model: UX 100 - 003 series).

2.2. Planting procedures and data collection

The one month old *in-vitro* potato plantlets produced from tissue culture laboratory were hardened and rooted in the screen-house before transplanting into the aeroponics unit. After 30 days, the plants were transplanted into the aeroponics. The first planting was done on 1st

November 2015 and the second in 1st December 2015. The plants were planted into holes on the styrofoam lid at the crown with roots hanging inside the box. The roots were fully exposed to the nutrient fog inside the growth chamber. The potato nutrient solution of Sichuan Academy of Agriculture Science, China was used. The nutrients were dissolved in distilled water and then poured into the nutrient tank. A 1000 L tank was used to store the nutrient solution for circulation and 0.75 HP constant pressures pump to distribute the nutrient solution through pipe. The solution was compressed through the nebulizers by a high pressure pump, forming a fine mist in the growth chambers, and which was sprayed onto the hanging potato roots. The plant roots received the nutrients from the nutrient mist and grew. The nutrient solutions were collected at the bottom of the growth chamber and flew back into the tank. This was re-used for 14 days, there after a fresh stock was added.

A simple random sampling method was used to select the sample plant for data collection. The sample numbers were determined by using Yamane (1967) formula with ± 5% precision level. The data were recorded from 12 tagged plants (N = 30) per plot at an interval of 15 days between each measurement (30, 45, 60, and 75 days after transplanting). Data on vegetative growth, yield and plant mortality rate were recorded. Plant height was measured with a calibrated ruler from the base of styrofoam to the plant apex. Average plant height per plant was calculated. Number of leaves per plant was recorded and articulated in numbers. Root length was measured from the stem suspended inside growth chamber to the tip. The percentage of plant mortality at harvest was determined. The total numbers of mini-tubers from 12 plant samples were harvested and mean per plant were calculated. The mini-tubers were categorized into two categories (i.e. normal weight > 8 g and underweight < 8 g) according to CIP evaluation (Otazu 2010). These grades were counted and weighed separately using digital weighing balance. The weight and number of mini-tubers were calculated for each treatment. The unit was recorded into weight (g per plants) and percent of mini-tubers under normal weight and underweight category. The average number of mini-tuber per plant was converted into number of mini-tuber per 4000 plants (the present capacity of AGH 306 m^2) for economic analysis.

2.3. Mean day and night temperature inside AGH

Plant growth rate and tuberization is influenced by day net photosynthesis and dark reaction (Leach et al 1982). Therefore, the day and night time temperature during the experimental period was assessed. The highest mean night and day time temperature was recorded in March (11.94 °C and 23.20 °C) and lowest (6.26 °C and 13. 81 °C) in January during the experiment periods (Figure 1). The three months (December, January and February) have relatively low temperature both for night and day (Figure 1), compared to recommended temperature (10 °C to 15 °C and 18 °C to 20 °C) respectively, for potato development and tuberization (Vandam et al 1996; Levy and Veilleux 2007; Otazu 2010). In case of two planting times (November and December), there was difference of 3 °C and 4 °C in the mean night and day temperature respectively.

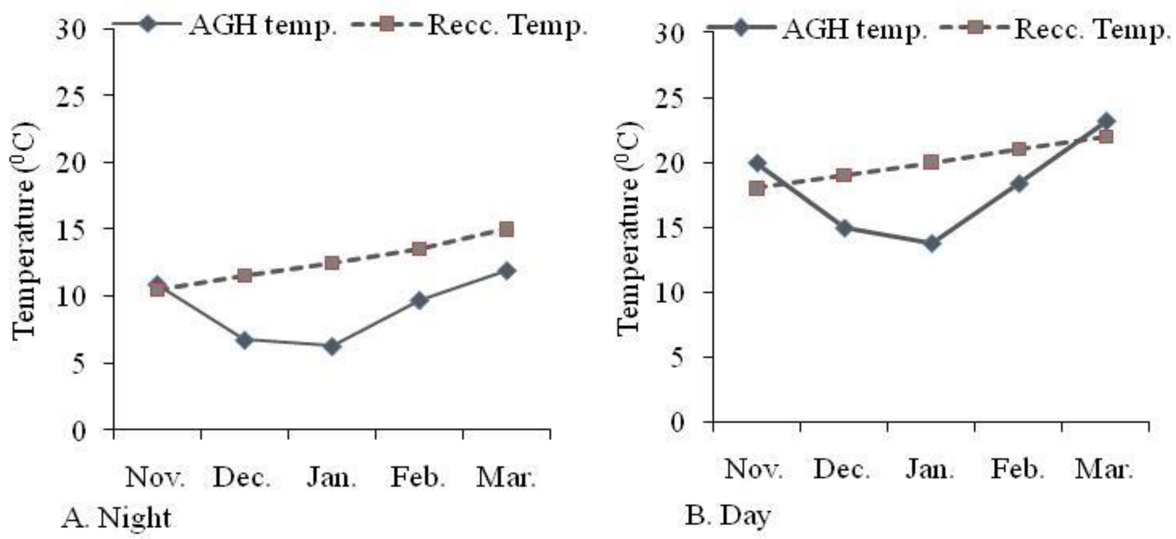

Figure 1. A. night and B. day, mean temperature inside Aeroponics Green House with recommended temperature

As per the SPSS guideline of Field (2009), the combined analysis (General Linear Model-Univariate) was performed to test the interaction between planting times and cultivars. Bonferroni multiple range test was used to test the level of significance among the means of the cultivars. Statistically the significant level was considered at $p = 0.05$.

3. Results and Discussions

3.1.Growth parameters

The combined analysis of variance showed highly ($p < 0.01$) significant differences between the three cultivars for plant height, number of leaves and root length (Table 1). However, the interaction effect of planting time and cultivars was not significant. The lack of interaction between planting time and cultivar effect was in contrast with the finding of Tshisola (2014); Farran et al (2006) that potato plant growth and number of leaves produced were subjective to environment and environment by genotype interaction. The insignificant effect of planting times in the present experiment could be due to the minimum temperature (3°C-4°C) difference between two planting time that did not permit strong competition between the plants. The early planting (October) need to be considered in future studies.

Table 1. Mean squares and significance levels from the combined ANOVA for plant growth

		Growth variables		
Source of variation	*df*	Plant height	No. of leaves	Root length
Planting time	1	5.89ns	7.45ns	6.57ns
Cultivars	2	440.96**	17718.71**	706.02**
Planting time * cultivar	2	2.35ns	0.813ns	0.23ns
Error	66	0.88	69.95	13.73

**Highly significant difference at P <0 .01; *df* = degree of freedom; ns = not significant

3.1.1. Effect of cultivars on plant height

Multiple comparison using Bonferroni test indicated that Nasephey Kewa Kaap showed tallest plant height (16.11 cm) compared to the lowest, Yusikaap (8.56 cm) at 75 days after transplanting (Figure 2). YK generally showed retarded growth throughout season under the aeroponics condition, however, there was no significance between the YK and KK. The highly significant difference among the cultivar for plant height might be attributed by genotypic differences. NKK responded positive under aeroponics condition than KK and YK. This result is in inline with the findings of Farran et al (2006) that the potato cultivar Zorba responded with higher vegetative cycle and plant height compared to other local cultivar under aeroponics system in Spain. Further, In Rwanda, potato cultivar Kinigi showed superior growth rate when compared to Kigega cultivars (Masengesho et al 2012). Farran et al (2006) stated that the inhibition (poor growth) was because of its weak capacity for utilizing the low light intensity in the aeroponics greenhouse. Therefore, this result indicates that plant height response to aeroponics system was cultivar dependent.

3.1.2. Effect of cultivars on number of leaves

The maximum numbers of leaves per plant were recorded in NKK plants (13, 29, 44, and 79) and the minimum in YK (7, 13, 18, and 32) at all the four consecutive measurements (Table 2). The significance difference due to cultivar was in an agreement with Otazu (2010) and Masengesho et al (2012) that the potato vegetative development inside an aeroponics is genotype dependent. Similarly, the study conducted in Iran by Movahedi et al (2012) reported that the Marfona cultivar had the highest numbers of leaves and root length compared to local cultivar, Agria and Savalan under aeroponics condition. Hence, the positive response of NKK might have triggered more number of leaves per plant. The least numbers of leaves in KK and YK might be attributed to poor growth under aeroponics system.

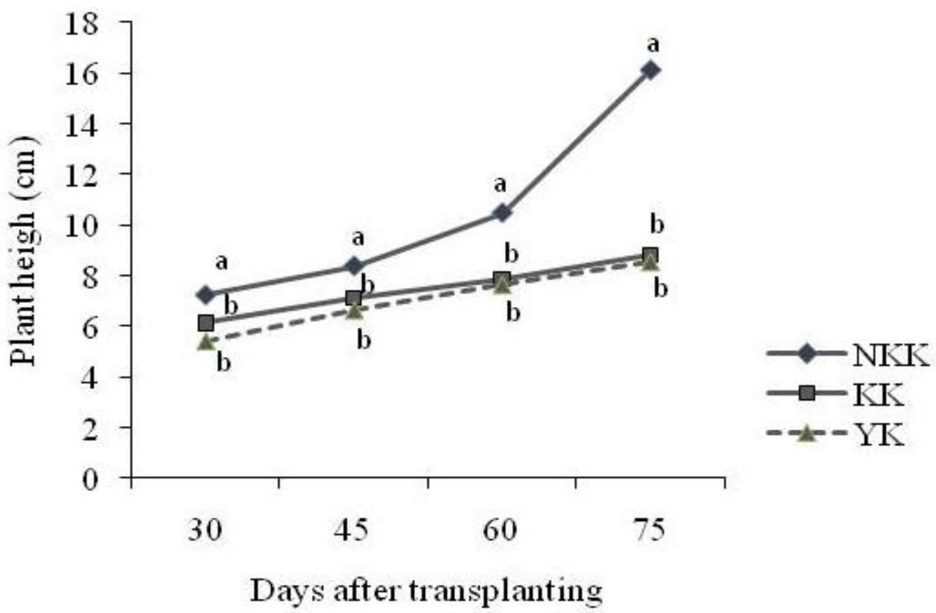

Figure 2. Effect of cultivar on plant height

Different letter (s) within DAT, indicates significant difference at $p < .01$ level

Table 2. Effect of cultivar on numbers of leaves per plant

Cultivars	Days after transplanting			
	30	45	60	75
KK	7[b]	12[b]	19[b]	32[b]
NKK	13[a]	29[a]	44[a]	79[a]
YK	7[b]	13[b]	18[b]	32[b]
F-value	**	**	**	**

** Highly significant difference at $P < 0.01$; different letter in column indicate significant difference

3.1.3. Effect of cultivars (cv) on number of root length

The mean root length showed high significant difference ($p < 0.01$) among the cultivars. The highest root length per plant was recorded in NKK (17.07 cm, 19.09 cm, 26.04 cm and 31.30 cm) and the lowest, YK (11.65 cm, 15.86 cm, 17.44 cm, and 21.34 cm) at all the measurements (Table 3). Conversely, there were no significant differences between KK and YK. As discussed earlier, the variation of root length was cultivar dependent and NKK being the tallest plant and with maximum numbers of leaves might have influenced better root development. According to Crist and Stout (1929), there is a persistent tendency towards a positive correlation between shoots, leaves and roots. The intensive studies in wheat plant showed that plants exhibited marked specific and varietal differences with respect to relative development of roots when grown under the same environmental conditions (Weaver et al 1924). The present study shows that the maintenance of a proper balance between root and shoot seems to be of great importance as reported by Weaver and Himmel (1929) that “if either is too limited or too great in extent, the other will not thrive”.

Table 3. Effect of cultivars on root length (cm)

Cultivars	Days after transplanting			
	30	45	60	75
KK	12.19[b]	16.27[b]	18.62[b]	22.60[b]
NKK	17.07[a]	19.09[a]	26.04[a]	31.30[a]
YK	11.65[b]	15.86[b]	17.44[b]	21.34[b]
F-value	**	**	**	**

** Highly significant difference at $P < 0.01$; different letter indicates high significant difference

3.2. Effect of planting time on plant survival

The records on plant survival of November and December planting were compared. The test showed statistically no significant difference between November and December timing, however, in absolute mean value, November planting time had the maximum (74%), $n = 270$, survival rate than December (68%), $n = 270$. The maximum survival rate could be due to higher night temperature (5°C) at the time of transplantation in November that favored the plant survival. Conversely, the lowest night time temperature (0.53°C) in December might have caused more injury to plants causing death. According to Patsalos (2005), at temperature of 3°C, serious damage is caused to the foliage and at temperature below minus 2 °C; plants freeze entirely and die.

3.3. Effect of cultivars on plant mortality

There were significant difference among the cv on plant mortality $F(2,3) = 13.73$, $p = .031$. The highest plant mortalities rate was recorded in YK (39%) and the lowest (16%) in NKK (Figure 3). However, the mortality rate of KK was not significantly different from both the highest and lowest rates. The highest rate of plant mortality could be due to low night time temperature weakening subsequent vegetative growth. Levitt (2005) reported that chilling injury results when plants are exposed at 0°C to 8°C for more than three hours. The rate of chilling (low night temperature) combined with weak plants could possibly have caused plant mortality. In contrast, the rate of low mortality could be due to sturdy plant growth and genotypic vigor. The cultivar with relatively long maturity periods grows better where temperature is a limiting factor (Mateus-Rodriguez et al 2014) such as in Peru, Huancayo (3,259 m.a.s.l.), chucmarina cv had shown low mortality compared to other CIP cv. Similarly, the late maturity cv, NKK (145 – 160 days) (DoA, 2015) had the highest number of survival rate than KK (100 – 120 days) and YK (100 – 120 days). This indicates that the rate of plant survival and mortality was also due to cv differences.

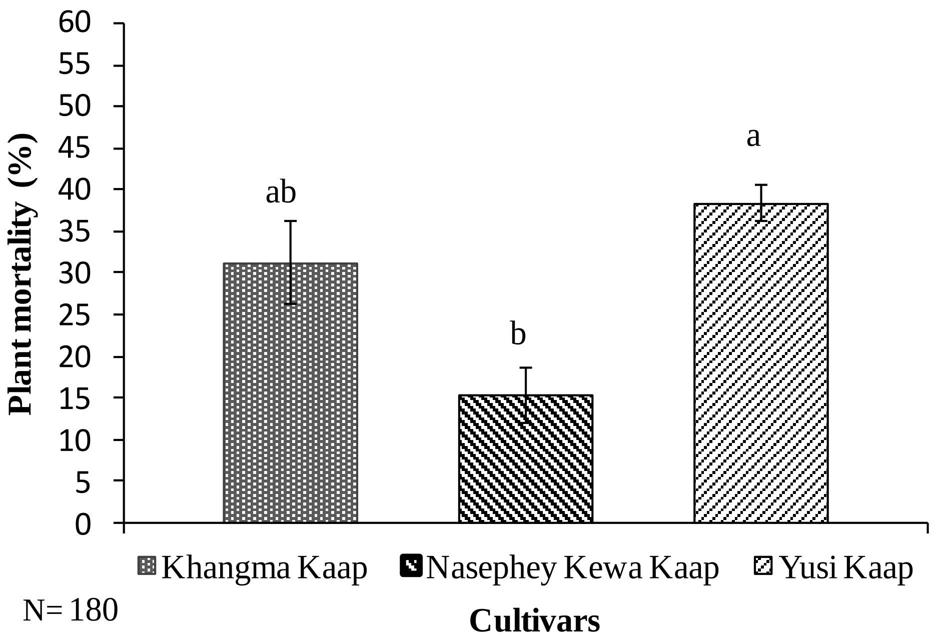

Figure 3. Percent of plant mortality

Different letter indicates significant difference at $p < 0.05$ level; Mean ± *SEM (standard error of mean)*

3.4. Yield parameters

3.4.1. Effect of planting time and cultivars on weight and number of mini-tubers per plant

The combined analysis of variance showed no significant differences due to effects of planting time and planting time by cultivars interaction in all the measurement of yield variables (Table 5). However, effect of cultivars showed highly significant difference ($p < .01$) for the four yield variables categories. This result indicates that the yield response under aeroponics was attributed by difference in cv traits and more testing of cv is recommended as explained by Otazu (2010).

Table 5. Combined ANOVA for the weight and number of mini-tuber per plant

		Mean square			
Source of variation	*df*	No. of mini-tuber per plant	No. of mini-tuber > 8 g	No. of mini-tuber < 8 g	Weight (g per plant)
Planting time	1	0.92ns	0.03ns	0.22ns	0.23ns
Cultivars	2	177.31**	3.16*	128.97**	747.78**
Planting time * cultivars	2	0.06ns	0.002ns	0.009ns	4.29ns
Error	66	1.32	0.18	1.78	5.26

*Significant difference at $p < .05$; ** highly significant at $p < .01$; ns = no significant difference

3.4.2. Yield and number of mini-tuber of three cultivars

The maximum yield (18.39 ± 0.213) gram per plant was recorded in NKK plot and the minimum (8.30 ± 0.564 g) from YK (Table. 6). The maximum yield recorded from NKK could be due to higher number of leaves and better plant growth. Similarly, Anand and Krishnappa (1988) reported that higher number of leaves and healthy crops yield high due to translocation of more photosynthates to tubers. The above result was in contrast with the finding of Tshoka et al (2012) that the low yields corresponded to an increase in above ground growth due to the competition for sucrose unloading between the storage organs (mini-tubers) and the above ground growth (leaves and stems). Such competition for assimilates controls aerial growth (leaves and stem) (Rykaczewska, 2004; Jackson, 1999). However, this study found that the maximum plant growth under aeroponics gave higher yield (grams per plant) compared to low vegetative growth.

Table 6. Effects of cultivars on yields

Cultivars	Yield (g per plant)	
	Mean	*SEM*
Khangma kaap	9.22[b]	0.21
Nasephey kewa kaap	18.39[a]	0.53
Yusikaap	8.30[b]	0.56
F value	**	

Different letter indicates significant difference at $p < .01$ level. ** Highly significant at $p < .01$

3.4.3. Number of mini-tuber per plant

NKK had significantly higher (8 ± 0.263) number of mini-tubers per plant compared to the lowest, YK (3 ± 0.212). Further, there was significant difference between YK and KK (5 ± 0.214) (Figure 4). The result indicates that cvs had a differential behavior for yield variables under aeroponics system. Similar to this study, Movahedi et al (2012) in Iran found one of the three varieties (Agria, Marfona and Savalan) performed better than the other two. Further, the study conducted in Rwanda by Masengesho et al (2012) and in Spain by Farran et al (2006) found that response in an aeroponics system is cv dependent and recommended to determine for each type. On the contrary, the study conducted in Hauncayo, Peru by Mateus-Rodriguez et al (2014) reported that yield variables were primarily influenced by genotype and environmental interaction. The more vegetative growth cv resulted in higher numbers of mini-tuber, as reported by Wolf et al (1990) that the increase in tuber number was due to increased photosynthetic activity and translocation of photosynthesis to the sink which have helped in the formation of more tubers.

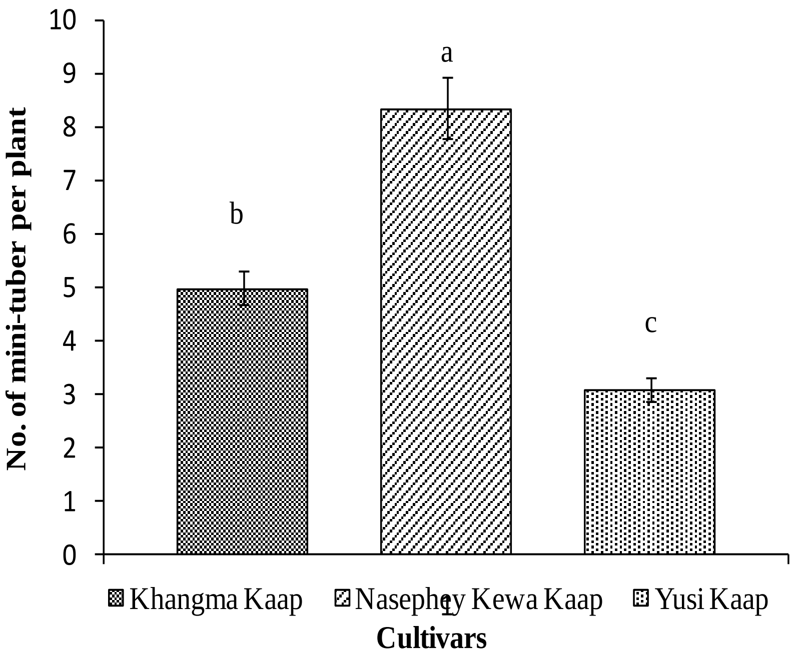

Figure 4. Effect of cultivars on numbers of mini-tubers per plant

Different letter indicates significant difference at $p < .01$; Mean ± *SEM*

4. Conclusion

There was no interaction due to effect of planting times and cultivars on plant growth and yield variables, but the main effect was due to cultivars. Planting time does not influence mini-tuber production for the measured traits of plant height, no. of leaves, root length, number of mini-tuber and weight. The cultivar effect was significant and Nasephey Kawa Kaap yielded highest both in terms of weight and no. of mini-tubers followed by Khangma Kaap and Yusi Kaap. NKK was found to be best performing and is recommended among the three cultivars for mini-tuber production through aeroponics. Since the response under aeroponics was cultivar dependent, further research is recommended to test more cultivars.

Acknowledgement

The author would like to thank the College of Natural Resource and National Seed Centre (NSC) for their support and facilities provided during the study. This study was possible only with the help of laboratory personnel of NSC, Paro. We pay our gratitude to Dr. Sonam Tashi, Dean of Academic Affair and Dr. Tulsi Gurung, Assistant Professor, CNR, Dr. Yadunath Bajgai, National Potato Coordinator, Mr. Wangda Dukpa, Program Director, NSC for their valuable suggestions and feed backs. We are also grateful to National Potato Program, Horticulture Division, DoA for the financial support.

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