

Full Length Research Paper

Longevity of Safed Musli (*Chlorophytum borivillianum*) tubers under different storage temperatures and concentrations of paclobutrazol

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Accepted 13 June, 2012

The effects of temperature and paclobutrazol (PBZ) in inhibiting tuber sprouting of Safed Musli during storage were determined. Tubers were stored at 28 and 10°C, each was sprayed with 0, 100, 200 and 300 mg/L PBZ weekly for 8 months. An uncontrolled precocious sprouting with excessive sprout growth of tubers was observed at 28°C without application of PBZ, while those treated with PBZ at 28°C had lesser precocious sprouting. Gradual increase in concentration of PBZ suppressed sprout growth. It was found that temperature played a vital role in sprouting inhibition as no precocious sprouting occurred in all treatments at 10°C in the present study. Tubers at 28°C recorded high respiration rate compared to those stored at 10°C for all concentrations of PBZ. Results also showed that low temperature was not only beneficial in inhibiting sprouting but also in maintaining the tuber quality for planting material. Tubers stored in 10°C showed better foliage growth compared to tubers in 28°C when planted in the field.

Key words: Sprouting, inhibitor, respiration rate.

INTRODUCTION

Safed Musli (*Chlorophytum borivillianum*) is a biannual herb which originates from India. It belongs to the family Liliaceae (Purohit et al., 1994). It is one of the most valued medicinal crop which has high potential to be used as an alternative to Viagra®, delay menopause and remedy for diabetes and arthritis (Manjunatha et al., 2008). The value of this crop lies in its tuberous roots used as natural aphrodisiac due to its active ingredient, the saponins. According to Raghavendra et al. (2005), there are more than 300 species under the genus *Chlorophytum*; however, Manjunatha et al. (2008) stated that Safed Musli or the species *borivillianum* is the species with the highest economic potential as it has high saponin content of 17%. To date, the market demand for the tuberous roots exceeds the supply, thus, increasing interest in the

production of this crop on a commercial scale. The crop is propagated using tuberous roots. Therefore, the tubers serve a dual agricultural function being the organ of economic importance as well as planting materials. Usually, Safed Musli tubers are harvested 9 months after planting and stored for at least 3 months while waiting for land preparation during the next planting season. The current practice for storage is by keeping Safed Musli tubers in a semi-open area (under shade) by placing it on thin layer of sand.

This method causes precocious tuber sprouting during storage even within a month after harvest. It has been reported that sprouting triggers degradation of biochemical content and the tuber quality (Trèche and Agbor-Egbe, 1996). Thus, the sprouted tubers cannot be stored for long. It has been reported that deterioration of harvested products is hastened in high temperature (Clark, 1994; Panneerselvam and Jaleel, 2008), especially in the tropics. In order to overcome this problem, many previous researchers attempted to store the

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postharvest products at low storage temperature (Paull, 1997; Osunde and Orhevba, 2009). In lily bulbets, high temperature resulted in total viability loss within 1½ years while storage at below 0°C retained more than 80% viability after 2 years of storage (Bonnier et al., 1996).

Although temperature offers a solution to control sprouting, other alternative methods need to be exploited especially when the required temperature cannot be achieved easily. In this context, Vliet and Schriemer (1963) proposed the use of sprouting inhibitor while maintaining the tuber at higher storage temperature. A potential chemical, which can be used in prolonging dormancy, is paclobutrazol (PBZ) (Hedden and Graebe, 1985). PBZ is a triazole compound categorized as plant growth retardant, and is mainly used to control vegetative growth of plants in various species. PBZ was reported to retard sprout elongation (Ranney et al., 1994; Wiesman and Lavee, 1994), while Lim et al. (2004) have shown that the use of PBZ was effective in controlling sprouting of two potato genotypes. Besides, Tekalign and Hammes (2004) also stated that potato from plants treated with PBZ and stored at room temperature had longer dormancy period over the control. The mode of action of PBZ have been reviewed by Hedden and Graebe (1985), where they discovered that PBZ interferes with gibberellic acid synthesis by inhibiting three steps in the metaloxin pathway which lowers the production of gibberellic acid essential for sprouting (Yamazaki et al., 2002). Many publications have suggested that the availability of optimum levels of endogenous gibberellic acid is the key factor in stimulating tuber sprouting (Majeed and Bano, 2006; Hartmann et al., 2011).

Therefore, the objective of this study was to prolong Safed Musli tuber storability by inhibiting tuber sprouting using two different temperatures and varying concentrations of PBZ.

MATERIALS AND METHODS

Safed Musli tubers were stored at two different temperatures, namely ambient temperature ($28 \pm 2^\circ\text{C}$) and cold temperature (10°C , using a cold room), in dark condition with $70 \pm 5\%$ relative humidity. In order to inhibit tuber sprouting, a commercial brand of growth inhibitor (CULTAR®, Syngenta Ltd.) which contained PBZ as the active ingredient was used. Tubers in each storage temperatures were sprayed with PBZ (0, 100, 200 and 300 mg/L) once per week for 8 months. Each treatment was replicated thrice with 40 tubers per replicate. Samples were removed at an interval of 2 months for data collection on percentage of precocious sprouting, sprout length, and respiration rate during storage. In order to test the viability of the tubers after the designated storage period, a sample of tubers from all treatments were first conditioned by incubation at 28°C for 2 weeks. Subsequently, tubers were sown in polyethylene bags.

The data obtained was subjected to the analysis of variance by Statistical Analysis System and means among treatments were compared using least significant difference (LSD) test at 5% level of probability. All data were analyzed separately upon sampling once in 2 months and mean comparison was made only for treatments within a particular storage period.

Percentage of precocious sprouting

Tubers which had sprouted more than 2 mm during storage were classified as having precocious sprouting. Percentage of precocious sprouting was taken every 2 months for 8 months.

Sprout length

The sprout length was measured from the tuber crown until the sprout tip by using graph paper and expressed in centimeters.

Detection of respiration rate

Carbon dioxide was detected using gas chromatograph (GC) (Clarus 500, Perkin Elmer, US) equipped with a flame ionization detector. Tubers were placed in the airtight container to trap gasses. The GC was switched on for an hour before use to stabilize the system. Trapped gas was sucked (1 ml) using syringe from the airtight container and injected into the GC.

RESULTS

Precocious sprouting

Sprouting percentage during storage for all treatments is shown in Table 1. Untreated tubers stored at 28°C , sprouted as early as 4 days in storage (data not shown) and by the end of 2 months 70% of the tubers had sprouted. With application of PBZ, only 35 to 45% sprouting was noted after 2 months of storage. In contrast, tubers stored at 10°C , showed no signs of sprouting irrespective of the concentrations of PBZ. By the 4th month of storage excessive sprouting had occurred with 91% of tubers kept at 28°C having sprouted, which clearly showed the negative effect of storage at room temperature. Higher concentration of PBZ (200 and 300 mg/L) had significantly less sprouting percentage compared to 100 mg/L. However, the effect of PBZ was minimal as compared to the effect of temperature; as the temperature strongly inhibited sprouting (Table 1), whereby even at 4 months of storage, no tuber sprouting was recorded except for 3% in control.

As the storage period progressed into 6 and 8 months, all concentrations of PBZ at 28°C did not show any significant difference in the inhibitory effect as the trigger to germinate has already been turned on. However, in the case of tubers stored at 10°C , minimal or no sprouting occurred from all treatments until 8 months.

Sprout length

Sprout length was measured in order to observe the pattern of sprout growth during 8 months of storage, and its comparison with tubers treated with PBZ. However, no measurement was taken for all tubers in 10°C as there was no sprout present throughout this study.

In the 2nd month after storage, control tubers in 28°C

Table 1. Percentage of tuber sprouting for 8 months of storage as affected by temperature and paclobutrazol (PBZ) application.

PBZ level (mg/L)	Storage period (months)							
	2 nd		4 th		6 th		8 th	
	Temp (°C)							
	28	10	28	10	28	10	28	10
0	70.3 ^a	1.8 ^c	91.7 ^a	3.3 ^d	91.8 ^a	3.3 ^c	91.9 ^a	3.3 ^c
100	45.3 ^b	0 ^c	71.9 ^b	0 ^d	73.6 ^b	0 ^c	73.6 ^b	0 ^c
200	36.9 ^b	0 ^c	65.1 ^b ^c	0 ^d	61.9 ^b	0 ^c	61.9 ^b	0 ^c
300	36.7 ^b	0 ^c	55.1 ^c	0 ^d	60 ^b	0 ^c	60.4 ^b	0 ^c
Prob.> F	10.64		10.92		13.77		14.11	

Data presented is analyzed individually for every sampling time of 2 months interval, where means with the same letter within the same group at different storage periods are not significantly different by LSD. PBZ and Temp indicated paclobutrazol and temperature, respectively.

Table 2. Sprout length (cm) during storage for 8 months as affected by temperature and Paclobutrazol (PBZ) application.

PBZ level (mg/L)	Storage period (months)							
	2 nd		4 th		6 th		8 th	
	Temp (°C)							
	28	10	28	10	28	10	28	10
0	5.13 ^a	0.1 ^d	8.3 ^a	0.1 ^c	9.43 ^a	0.2 ^d	9.67 ^a	0.2 ^e
100	1.67 ^b	0 ^d	2.63 ^b	0 ^c	3.30 ^b	0 ^d	3.70 ^b	0 ^e
200	1.53 ^b	0 ^d	1.97 ^{bc}	0 ^c	2.37 ^c	0 ^d	2.43 ^c	0 ^e
300	0.97 ^c	0 ^d	1.37 ^c	0 ^c	1.57 ^c	0 ^d	1.63 ^d	0 ^e
Prob.> F	0.51		0.67		0.81		0.65	

Data presented is analyzed individually for every sampling time of two months interval, where means with the same letter within the same group are not significantly different by LSD. PBZ and Temp indicated paclobutrazol and temperature, respectively.

had the longest sprout measuring 5.13 cm (Table 2). Tubers treated with lower concentration of PBZ (100 and 200 mg/L) showed lower sprout length (1.67 and 1.53 cm, respectively), with no significant difference between them. On the other hand, high concentration of PBZ (300 mg/L), inhibited sprout growth resulting in sprout length of only 0.97 cm. With increased time in storage, at 4 months, tubers kept in 28°C had faster sprout growth compared to other treatments, giving an average length of 8.3 cm. Data in Table 2 revealed that treatment with 200 mg/L of PBZ showed no significant difference compared to 100 and 300 mg/L of PBZ. But, significant difference was found between 100 and 300 mg/L, with 100 mg/L PBZ having 1.26 cm longer sprouts compared to 300 mg/L. Thus, the increase in PBZ concentration resulted in higher suppression of the sprout growth in Safed Musli tubers. Surprisingly, during this 4 months of storage, 20% of control tubers stored at 28°C flowered, had blackening of sprout, begun to shrink and was dehydrated (Figure 1). However, such events did not occur for tubers treated with PBZ at the same temperature.

On the 6th month after storage, non-treated tubers in 28°C showed only a small increase in sprout length (1.3 cm) from the 4th month after storage (Table 2). This might be due to the tubers having attained its maximum shoot length. Although, there was only a small increase, but it was still longer compared to the treated tubers. Concentration at 200 and 300 mg/L showed the lowest sprout elongation (2.37 and 1.57 cm, respectively), compared to 100 mg/L (3.3 cm) with similar effect at 8 months after storage (Figure 2).

Respiration rate

During the 2nd month of storage, the respiration rate for tubers stored in 28°C was eight times higher, compared to tubers stored at 10°C. The effect of reducing temperature on respiration rate can be clearly observed for all treatments of Safed Musli tubers stored at 10°C (Table 3) with the rate of respiration being less than 1.3 mg CO₂/kg/h. However, control tubers in 28°C recorded

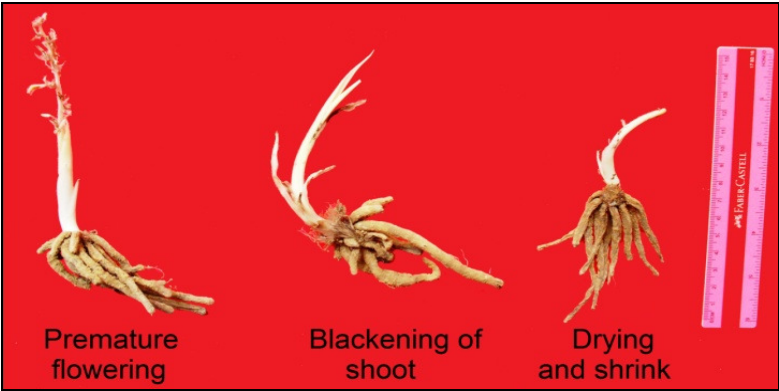


Figure 1. Some of the control tubers in 28°C had advanced sprouting on the fourth month after storage with three abnormal events occurring after 4 months of storage.

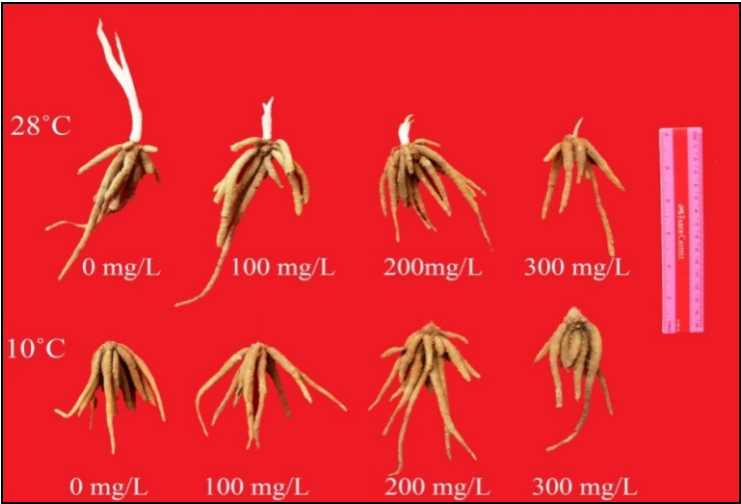


Figure 2. Sprout growth as affected by the concentrations of PBZ at 8 months after storage. Control in 28°C had the longest sprout length and the sprout length was suppressed with increasing concentration of PBZ. No sprout was present when stored at 10°C.

Table 3. Respiration rate (mg CO₂/kg/h) during 8 months of storage as affected by temperature and paclobutrazol application.

PBZ level (mg/L)	Storage period (months)							
	2 nd		4 th		6 th		8 th	
	Temp (°C)							
	28	10	28	10	28	10	28	10
0	11.80 ^a	1.22 ^d	13.31 ^a	2.90 ^d	10.07 ^a	1.5 ^d	5.96 ^a	1.42 ^c
100	9.40 ^b	1.17 ^d	10.17 ^b	2.77 ^d	8.91 ^b	1.41 ^d	4.45 ^b	1.32 ^c
200	7.43 ^c	1.11 ^d	9.79 ^{bc}	2.72 ^d	8.13 ^{bc}	1.32 ^d	4.02 ^b	1.27 ^c
300	6.94 ^c	0.84 ^d	9.13 ^c	2.32 ^d	7.34 ^c	1.24 ^d	3.99 ^b	1.1 ^c
Prob.> F	1.15		0.99		0.88		0.86	

Data presented is analyzed individually for every sampling time of two months interval, where means with the same letter within the same group are not significantly different by LSD. PBZ and Temp indicated paclobutrazol and temperature, respectively.

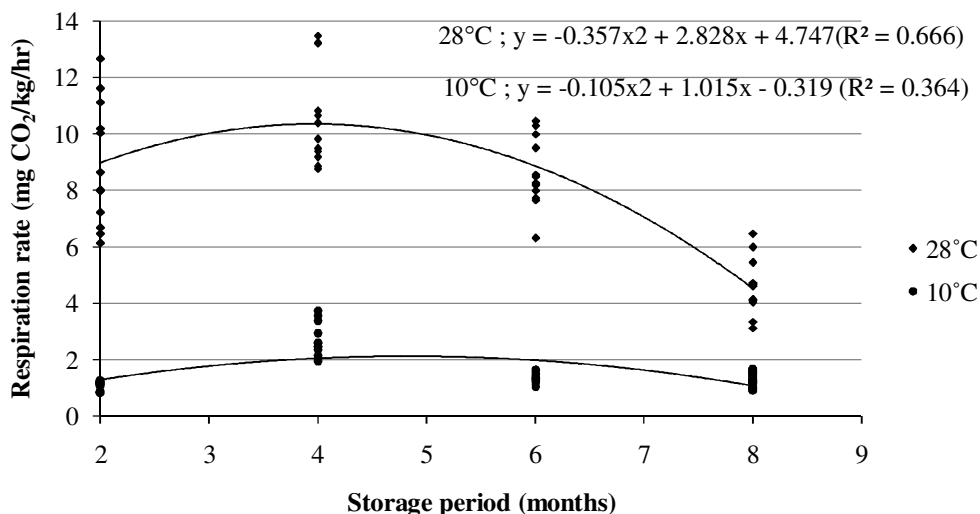


Figure 3. Regression analysis on the respiration rate for tubers stored at two different temperatures which were 28 and 10°C for 8 months.

the highest respiration rate (11.8 mg CO₂/kg/h) compared to treated tubers in the same temperature. Tubers treated with 100 mg/L of PBZ at 28°C had slightly lower respiration rate which is 9.4 mg CO₂/kg/h, while no significant difference was found between treatment of 200 and 300 mg/L of PBZ (7.43 and 6.94 mg CO₂/kg/h, respectively).

On the 4th month of storage, tubers in all treatments both stored at 28°C as well as 10°C, showed an increase in the respiration rate with control in 28°C having the highest respiration rate (13.31 mg CO₂/kg/h). If mean values are used it can be seen that at 28°C the mean respiration rate increased from 8.89 at 2nd month of storage to 10.6 at the 4th month. While for 10°C it increased in mean value from 1.08 to 2.68 at 2 and 4 months, respectively. As temperature had stronger effect compared to PBZ in inhibiting sprouting, a regression between two temperatures was made to predict the peak period of the respiration rate (Figure 3). Based on the regression equation, tubers in 28°C reached maximum respiration rate on 4th month, while tubers in 10°C reached a maximum at 4.8 months. Although the difference is minor (around three weeks), the respiration in 28°C is five times higher compared to 10°C. On the 6th month after storage, all treatments in both temperatures showed a decline in the respiration rate, especially in 28°C (Table 3). However, the high respiration rate was still recorded from control tubers at 28°C with 10.07 mg CO₂/kg/h, followed by 100, 200 and 300 mg/L of PBZ. Similar pattern with the previous months was observed for 200 mg/L where it is not significantly different with both 100 and 300 mg/L of PBZ at 28°C.

In the case of 10°C, a small decrease in respiration rate was noticed from 6 to 8 months after storage (1.37 to 1.28 mg CO₂/kg/h). As the respiration rate is very low, it raises two possibilities in this study, either the tubers had

slower respiration rate to maintain the dormancy or the cells are dead due to the low temperature. In order to answer this question, tuber viability test was carried out.

Tuber viability

It was found that tubers from 10°C storage, which remained dormant during the storage period started to sprout when they were planted. After 2 months of storage, 83% (Table 4) of the tubers sprouted. A slight decline in sprouting percentage was observed after 8 months of storage with 72% sprouting. Tubers stored at 28°C, which had already sprouted prior to planting were able to continue growth and development, thus, having expanded leaves very early after sowing. It was noticed that tubers from all treatments had no significant difference amongst them where the sprouting percentage was at 84 to 86% for tubers from the 2nd month storage period (Table 4). Extended storage duration up to 8 months also did not affect the ability to sprout especially in relation to the effect of temperature. However, increasing the concentration of PBZ lowered sprouting percentage (circa 70% sprouting percentage). The term sprouting used here and the percentage of sprouting shown in Table 4 refers to all tubers (sprouted and non-sprouted) which were able to establish itself into a plant without dying. Therefore, the data on sprouting may not give the expected impression on the response to the different storage temperature.

Based on morphological characteristics of the plants, the effect of storage environment, in this case being temperature, can be clearly distinguished. Plants originating from different storage temperature differed in number of leaves, leaf size and senesced much faster as compared to tubers that had not sprouted in storage (Figure 4).

Table 4. Sprouting percentage upon removal from the storage as affected by different storage temperatures and application of PBZ.

	Storage period (months)			
	2 nd	4 th	6 th	8 th
Temperature (T)				
28°C	85.2	82.6	80.8	72.6
10°C	85.8	82.6	80.8	77.1
Prob.> F	3.32	3.39	4.23	5.51
Paclobutrazol (P)				
0 mg/L	88.3	85.9	81.9	81.9a
100 mg/L	83.7	82.4	82.3	75.4ab
200 mg/L	84.2	81.8	81.2	71.7b
300 mg/L	85.6	80.4	77.9	70.5b
Prob.>F	4.69	4.79	5.98	7.79
T × P	ns	*	ns	ns

Data presented is analyzed individually for every sampling time of two months interval, where means with the same letter in a same column is not significantly different by LSD. PBZ, Temp, * and ns indicate paclobutrazol, temperature significant and non-significant, respectively.

**Figure 4.** Safed Musli plants from four months old tubers. Plants from tubers stored at 28°C with poor foliage growth compared to plants from tubers stored at 10°C.

This can be clearly seen starting from the 4th month after storage onwards.

DISCUSSION

This research has revealed that precocious sprouting of Safed Musli tubers was strongly influenced by the storage temperature, with minimal effect from the application of PBZ. This can be seen at 10°C where no sprouting occurred in both treated and untreated tubers. In 28°C, the concentration of PBZ itself did not have significant effect on storage of tubers but the presence of PBZ was necessary to suppress sprouting. The use of PBZ had little inhibitory effect on storage especially when lower concentrations were utilized. The failure of PBZ to

inhibit all tubers from sprouting might be due to the level of endogenous gibberellic acid in the tubers which was already high at harvest to result in initial sprouting prior to PBZ application.

Sprout growth and elongation after sprouting event during storage is mainly dependent on the amount of gibberellic acid. It has been reported that large quantity of gibberellic acid was detected during rapid sprout elongation, mainly through the synthesis via the mevalonic pathway (Hill, 1980; Arteca, 1996). Hence, elongation appears to be a factor of GA₃ synthesis de novo. Although biochemical determination of gibberellic acid content in the sprouting tubers was not carried out, but based on the sprout morphology in PBZ treated tubers, this hypothesis appear to be true as PBZ treated shoots were much shorter due to interference in the

mevalonic pathway thus, limiting gibberellic acid biosynthesis. The mentioned phenomenon is supported by the observation that increasing PBZ concentration resulted in higher suppression in the sprout growth of Safed Musli tubers. This finding was in agreement with Wiesman and Lavee (1994) who mentioned that, potato sprout growth was slower compared to those treated with higher concentration of PBZ.

As shown earlier, some abnormal events occurred in control tubers stored at 28°C namely early flowering, blackening of sprout and shrunken tubers. Such event clearly indicates that various hormonal inductions have taken place due to the stressful conditions of storage. However, such abnormal occurrence was not present for tubers treated with PBZ in the same temperature. Based on this, it is speculated that although PBZ is less effective in inhibiting spouting, but it was beneficial in a way where it prevented enhanced physiological ageing from occurring. Besides, control tubers at 28°C also exhibited exhaustion of storage carbohydrates which was identified by the growth of sprout. The sprout growth which was fast at the beginning gradually slowed down when it approached 8 months of storage.

Under normal growing conditions in the field, sprout growth and development at the initial stage is supported by the storage reserves from the tubers, but the initiation of roots as early as 15 days after sprouting also indicate that roots are beginning to function and contribute towards further development. However, in the case of sprouting in storage, the sprout growth is fully dependant on the storage reserves inside the tubers as further development such as root formation cannot occur in storage due to unfavorable condition for growth. This will result in rapid loss of storage reserves. Scott (2008) who has reviewed the relationship between sprout and root growth stated that, sprout growth must be supported by the root to supply mineral and water to prevent great damage during the plant growth and development. As various events take place during sprouting, the presence of sprout, however, may affect the plant growth and development in field as sprouting has been triggered, utilizing much of the reserves in the tubers.

As temperature and PBZ has impact on the storage of tubers, respiration rate was detected to understand the physiological changes that occurred during these 8 months of storage. It was found that respiration rate was positively correlated with both, sprouting and sprouts growth. A review by Pringle et al. (2009) stated that the availability of carbon dioxide in the environment was able to accelerate sprouting compared to an environment with low carbon dioxide. This phenomenon was evident in this study where excessive precocious sprouting in control tubers occurred on the 4th month after storage concomitant to the increase in respiration rate. High respiration rate may be the cause for the abnormal events observed during storage. High respiration rate often cause an excessive water loss, which lead to tuber

shrinkage. While in relation to the blackening to sprouts, early researcher have stated that the increased of carbon dioxide content in the storage vicinity can cause blackening of sprout tips (Thornton, 1935). The results clearly demonstrated the influence of temperature in controlling sprouting in Safed Musli. Increasing in respiration rate was noticed since the beginning of storage period and was then found to be declined after 4 months of storage, especially in tubers stored at 28°C. Indeed, higher respiration rate may result in hastening the degradation processes. A rapid degradation of storage carbohydrates occurred in tubers stored at 28°C, in particular, of control tubers in 28°C. Tubers respire strongly at higher temperature where it release large amount of CO₂, but only to a certain point, thereafter the respiration becomes very intense (Rastovski, 1987). The tuber cells can no longer obtain oxygen and the carbon dioxide produced cannot be released thus, the cells will gradually die. The low respiration rate during storage is advantages as this will ensure that the tubers have longer shelf life compared to tubers which had higher respiration rate (Ghazawi and Houshmand, 2010). Although the respiration rate declined from 4th to 6th month after storage, it was observed that there is no significant difference between these 2 months. Irrespective of PBZ concentrations, the respiration rates of all treated tubers at 28°C were not significantly different with each other at 8 months of storage. This is unexpected as the sprout growth differed among the treatments. Thus, it is assumed that all tubers respired at a minimum rate during prolonged storage, where it is sufficient for the tubers to maintain its viability and sprout growth. The ability of tubers to sprout after being stored for a long period is an important factor in establishing a proper storage system for Safed Musli. There is a possibility that due to cold temperature; secondary dormancy may have set in. However, this can be determined if the duration in storage was extended and biochemical means of viability test is carried out.

It is only logical that due to advanced physiological age of the sprouted tubers, plants from tubers stored at 28°C were a few stages ahead of plants from 10°C thus, resulting in early senescence. Wiersema and Cabello (1986) mentioned that using older tubers as the planting materials can enhance faster growth and development, reach maturity faster but this can also impair yield significantly. As the tuberisation is mainly dependent from the source (foliage), poor development of leaves may result in reduced yield (Wiersema and Cabello, 1986). It is also related to having a much shorter time with sufficient photosynthetic activity for tuber bulking as the plants senesced faster. It is also important to mention that not only yield may be affected but if the resulting tubers are to be used as planting materials for the following season, there is a high possibility that crop will fail due to tuber quality.

Different concentrations of PBZ had not affected the

foliage development. The difference on the foliage development between 28 and 10°C may be related to the amount of available storage reserve in the tubers. It was established that due to higher respiration rate, the rate of utilization of the storage reserve increased. Upon transfer to the field, especially at the initial stage while waiting for roots to be induced and function, the plant will have to depend on the limited resources thus, affecting its further growth and development in an effective way.

This study has clearly shown that in order to maintain tuber viability of Safed Musli during storage, the tubers have to be stored at low temperature. Storage at 10°C retains tuber viability for about 8 months with comparable viability to fresh tubers.

ACKNOWLEDGEMENT

The authors are grateful to Yayasan Felda for financing this work through a research grant and Universiti Putra Malaysia for all the facilities to successfully carry out this work.

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