Correlation between true seed and tuber dormancy in a *solanum tuberosum* group phureja × stenotomum population

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Abstract

Short tuber dormancy is desirable when multiple cropping seasons occur yearly, yet most potato cultivars were developed for regions with one growing season per year and have long dormancy. Although several methods exist for breaking tuber dormancy, they can lead to uneven emergence and poor stand counts. Breeding short dormancy cultivars could alleviate this problem. A long-day adapted diploid hybrid population of *S. tuberosum* Group *phureja*-*S. stenotomum* (*phu-stn*) has been developed with short tuber dormancy. The purpose of this study was to determine if there was a correlation between tuber dormancy in these *phu-stn* parents and true potato seed dormancy in their offspring, which would allow rapid breeding of short-dormancy germplasm. Tuber dormancy was evaluated for 12 diploid *phu-stn* parents harvested from three different locations: Presque Isle, Maine (ME), Plymouth, North Carolina (NC) and Beltsville, Maryland (MD) and stored at 7 °C in MD. Twelve crosses were made among these parents. Each parent was represented twice in the offspring. True potato seed (TPS) from these 12 families were disinfected and soaked in water for 24 hours, dried, and sown in tissue culture. TPS germination was recorded daily for 35 days and the germinated proportion was calculated. The experiment was conducted three times. Parental tuber dormancy ranged from 6-10+ weeks and varied significantly among locations. TPS family proportion germination ranged from 9 to 99%. There was no correlation ($r = 0.01$) between parental tuber dormancy and seedling vigor index. These results show that the relationship between offspring TPS dormancy and their parent's tuber dormancy is unpredictable. Additional research is needed to determine if selection for early sprouting in the offspring *in vitro* is correlated with short tuber dormancy in subsequent field generations of these offspring.

Keywords

Park, Conflict, Food Security, Participation, Nepal

1. Introduction

Potato has been propagated asexually by tubers. However, true potato seed i.e. botanical seed has potential in many tropical and subtropical areas as high quality tubers are difficult to produce in warmer regions, because of tuber-borne pathogens especially viruses. Pakistan is blessed with different agro-climatic zones (Malagamba, 1988)[1-4]. Farmers are raising two crops of potato namely autumn and spring while the third is grown in hilly areas during the summer in

plain areas. As a result of the short time between harvesting and planting of consecutive crops, farmers have adapted autumn-autumn cycle resulting in poor and expensive crop and seed production. Seed potato from summer crop have only been used as seed to the spring crop for seed multiplication and in autumn for further carrying over autumn to autumn cycle. Hence, short time gap between planting and harvesting of consecutive crops instigate farmers adapt autumn to autumn cycle being the only national potato seed strategy for Pakistan. It would be of great value for potato growers to develop early maturing and short-dormant varieties so the seed could be rotated around from between the crops[5-7].

Currently, in Pakistan a few Dutch-origin potato varieties are grown, have moderate to deep dormancy (Ahmed and Bhutta, 1995); and need to be stored at cold temperatures to enable next planting. But the country does not have enough cold storage facilities. Actually, these cultivars have been developed according to their regional conditions with one growing season per year. Development of early maturing varieties with short dormancy would be beneficial for growers as due to lack of appropriate material, the dormancy breaking methods give uneven emergence and poor stand counts. Unfortunately, issues related to tuber dormancy are rarely investigated in breeding programs[8-10].

Dormancy in potato is an inherent character and characterized as “the incapability of a seed/ tuber to germinate/ sprout in a specific period of time even under favorable environmental factors i.e. temperature, light/ darkness etc. (Baskin and Baskin, 2004; Claassens et al., 2005). Reust and coworkers (2001) studied some attributes while selecting short-generation seedlings comprising days to germination, physiological vigor/ rusticity and incubation period. These attributes may possibly be helpful for making selections for short-growing season potato crop. Seed vigor is a principal indicator of seed quality and involves rapid and uniform emergence of normal seedlings within a particular period (Zhu and Hong, 2008). “Physiological vigor/ rusticity” of a seed tuber is measured as the capacity of a clone having its 80% of tubers sprouted at least 2 mm long (Pande et al., 2007). The “incubation phase” is the period between the onset of sprouting and tuber initiation on the developing sprouts (Madec and Perennec, 1962). Seed dormancy in potatoes, has been positively correlated with tuber dormancy (Simmonds, 1964). So, the quality seed (TPS) can not only be related to final yields, but might also affect seed tuber quality (Barrington, 1971)[11-15].

Potato breeding program have a first generation of tuber production from botanic seeds, followed by generations of clone selection in the field (Bisognin, 2003). A large number of clones might need to be discarded, since the first generation because of limited number of tubers production having deep dormancy (Bisognin et al., 1998). Since, the dormancy is a character expressed very late. If this character can be correlated with some early expressive characters, much time can be saved by accurate measuring those indirect characters. The seedling emergence in tissue culture and dry matter contents are positively correlated with tuber dormancy and predominately additive in nature (Rashid and Carpena, 1997)[15-17].

The cultivated tetraploids can probably be promising for the highest yield but the diploids in comparison are gaining popularity, having more potential than is yet appreciated (De Maine, 1996). The tetrasomic inheritance of tetraploids also makes potato breeding programs complicated (Iwanaga, 1980). On the other hand, selection progress is more rapid with diploids due to simple disomic inheritance as increasing gene frequency at the 4x level is slower than at the 2x level (Iwanaga, 1980; Jansky, 2009). The hybrid population *S. phureja* × *S. stenotomum* was developed in the USA for heat tolerance, adaptation to long days, tuber appearance and high dry matter (Gautney and Haynes, 1983; Haynes and Haynes, 1983). They have gained a significance for their colorful flesh containing higher amount of carotenoids (Brown et al., 2006) and resistance to several diseases i.e. early blight (Christ and Haynes, 2001), resistance to soft rot *Erwinias* (Wolters and Collins, 1995), bacterial wilt and late blight (Costanzo et al., 2005). The genetic variance for tuber dormancy is very high in *S. phureja* and *S. stenotomum* populations (Thompson et al., 1980). So measuring seed dormancy and its correlation with tuber dormancy will be expedient for breeders minimize clone discard, due to either no or late plantlet emergence. The diploid potatoes having chosen characteristics are selected afterwards for the production of tetraploid hybrids through bilateral (BSP) and unilateral (USP) sexual polyploidization. BSP encompasses the cross between the female parent (forms 2n eggs) and the male parent (forms 2n pollen). In contrast, USP involves the cross between the parents producing 2n gametes (either 2n pollen or 2n eggs). The manipulation of 2n gametes and the asexual reproduction system of the potato provide the breeders the unique possibility of accumulating non additive genetic effects at the tetraploid level of the offspring. The objective of the current study is to determine tuber dormancy in a random sample of diploids, and determine if tuber dormancy in the parents is correlated with seedling dormancy in their offspring[17-21].

2. Material and methods

The 12 random crosses were selected for this study and 40 seeds from each cross were sterilized and soaked in double distilled water. After drying them for 24 hours, one seed was put in each tissue culture tube containing media (**Table 1**) and labelled with family ID. The temperature and humidity were 19 °C and 51%, respectively, in tissue culture room. All the tubes were arranged in racks and kept in the growth room. At the time of germination, each genotype was assigned a genotype number in order of germination i.e. WHD 1-1, WHD 1-2, etc. Plantlets were transplanted in greenhouse when there were two sets of true leaves. The tubers of parental genotypes were harvested from three different locations Presque Isle, Maine; Plymouth, North Carolina and BARC, Maryland. The tubers were kept in cold store of BARC, Maryland, USA at 7 °C and analyzed weekly for sprouting data.

Table 1. The composition of medium used for growing seeds

No.	Ingredients	Concentration
1	H ₂ O (Double distilled)	1L
2	Thiamine-HCl (0.04 g/ 100 ml DD H ₂ O)	1 mL
3	MS Basal salts	4.33 g
4	Inositol (Myo-Inositol)	0.10 g
5	Sucrose	20 g
6	Na H ₂ PO ₄	0.17 g
7	1N NaOH	1.3 mL
8	PPM	1 mL
9	Phytigel	1.57 g

2.1. Study of true seed dormancy

2.1.1. Germination (%)

The number of germinated seeds was recorded on a daily basis and their percentage was calculated.

2.1.2. Average seedling length (cm)

The seedling height was taken at 14th and 21st day from every culture tube and their average was calculated.

2.1.3. Seedling vigor index

Vigor index was calculated as $VI = S \times \Sigma (Gt/Dt)$ (Zhu and Hong, 2008); where S is seedling height of the 14th day, Gt is number of germinated seeds in the “tth” day, Dt is number of days from the first day to the “tth” day.

2.2. Study of tuber dormancy

2.2.1. Dormancy duration (days)

Dormancy duration was counted as number of weeks from harvesting to sprouting when 80% of the tubers (24 tubers for each genotype), with dormancy considered ‘broken’ when sprouts reached 2 mm in length (Pande et al., 2007). Tubers were scored daily for dormancy.

2.2.2. Sprouting (%)

Sprouting percentage was calculated as percentage of sprouted tubers over total number of tubers. It was determined at the end of experiment when approximately all the tubers had visible sprouts.

2.3. Statistical analysis

For the statistical analysis, the experimental data were subjected to analysis of variance (ANOVA) using Statistix 9[®] for Windows (Analytical Software, Tallahassee, USA). Fisher's least significant difference (LSD) was used following a

significant ($P < 0.05$) F-test. Correlation was analyzed by the general linear models procedure in SAS, version 9.2 (Cary, NC) as described (Anjum et al., 2018).

3. Results

3.1. Study of true seed dormancy

3.1.1. Germination (%)

The genotypes differed significantly for germination percentage. The seeds of genotype WHD-7 had maximum germination percentage, statistically similar to that of WHD-4 seeds. In contrast, seeds of genotype WHD-6 did not germinate at all (Table 2).

3.1.2. Average seedling length (cm)

Significant variations in seedling length were observed among genotypes. The genotypes WHD-4, WHD-7, WHD-8 and WHD-9 were found to have the longest seedlings which were statistically comparable with each other (Table 2). The smallest seedling was observed in WHD-6.

3.1.3. Seedling vigor index

The genotypes had significant differences for SVI. The highest SVI was noted in genotypes WHD-4 and WHD-7 and lowest in WHD-6 following WHD-1, WHD-12, WHD-3 and WHD-2 (Table 2).

Table 2. Mean comparison of true seed germination percentage, seedling length and seedling vigor index in 12 potato genotypes.

Genotypes	Parents (Female $\times$ male)	Germination %age	Average seedling length (cm)	Seedling vigor index
WHD-1	BD1301-3 $\times$ BD1315-3	5.83fg	14.11b	3.10f
WHD-2	BD1306-4 $\times$ BD1335-4	15.83ef	12.32b	6.89ef
WHD-3	BD1306-4 $\times$ BD1341-5	13.33f	14.14b	6.76ef
WHD-4	BD1308-3 $\times$ BD1330-3	90.83ab	17.68ab	53.56a
WHD-5	BD1311-4 $\times$ BD1310-1	30.83d	13.94b	11.94de
WHD-6	BD1311-4 $\times$ BD1334-2	0.00g	0.00c	0.00f
WHD-7	BD1312-5 $\times$ BD1334-2	94.17a	17.88ab	49.05a
WHD-8	BD1315-3 $\times$ BD1310-1	50.83c	21.01a	31.50c
WHD-9	BD1330-3 $\times$ BD1308-3	82.50b	17.77ab	40.97b
WHD-10	BD1341-5 $\times$ BD1301-3	25de	14.68b	11.62de
WHD-11	BD1421-5 $\times$ BD1312-5	32.5d	14.59b	16.11d
WHD-12	BD1421-5 $\times$ BD1335-4	9.16fg	13.48b	3.85f
LSD G ($P \leq 0.05$)	10.367	6.050	7.564	

Treatment means followed by the same letter are not significantly different. LSD = Least significant difference.

3.2. Study of tuber dormancy

The dormancy period and sprouting percentage of tubers from 13 *Solanum phureja* $\times$ *S. stenotomum* hybrids grown at three different sites in USA was determined immediately after harvest.

3.2.1. Dormancy duration

Statistically significant differences in dormancy duration were observed under main effects of location and genotype and their interaction (Table 3). The tubers harvested from Maine and Beltsville had shorter tuber dormancy than had the North Carolina tubers. The longest dormancy duration was observed in tubers of genotypes BD1310-1, BD1335-4, BD1315-3 and BD1326-1 whereas the genotypes BD1308-3 and BD1301-3 had the shortest tuber dormancy. The genotypes BD1312-5, BD1334-2 and BD1306-4 did not have their tuber dormancy break at all.

Table 3. Tuber dormancy duration and sprouting percentage of 13 parental genotypes from three different locations.

Factors		Dormancy duration (Weeks)	Sprouting %age
Location (L)	Maine	8.15b	81.43a
	Beltsville	8.38b	81.30a
	North Carolina	8.76a	70.46b
LSD L ($P \leq 0.05$)		0.231	1.227
Genotype (G)	BD1310-1	9.00a	88.35c
	BD1334-2	NDB	43.11g
	BD1306-4	NDB	32.28h
	BD1326-1	9.00a	79.24d
	BD1308-3	6.33d	93.58b
	BD1335-4	9.00a	76.64e
	BD1301-3	6.67cd	100a
	BD1330-3	7.00c	90.58c
	BD1311-4	7.00c	89.05c
	BD1315-3	9.00a	66.31f
	BD1341-5	7.00c	95.08b
	BD1421-5	7.67b	89.15c
	BD1312-5	NDB	67.17f
LSD G ($P \leq 0.05$)		0.481	2.553
LSD L $\times$ G ($P \leq 0.05$)		1.353	4.423

Treatment means followed by the same letter are not significantly different. LSD = Least significant difference. NDB = No dormancy break.

The maximum tuber dormancy was recorded in genotypes BD1310-1 and BD1330-3 harvested from any experimental location and BD1326-1, BD1315-3, BD1335-4 and BD1421-5 harvested from the Maine and Beltsville. The shortest tuber dormancy was observed in BD1308-3 and BD1341-5 from any experimental location, BD1301-3 from the Maine and Beltsville and BD1311-4 from the Maine and North Carolina (**Table 4**).

Table 4. Mean comparison of tuber dormancy duration of under the interactive effect of location $\times$ genotype.

Genotype	Location		
	Maine	Beltsville	North Carolina
BD1310-1	9.00a	9.00a	9.00a
BD1334-2	NDB	NDB	NDB
BD1306-4	NDB	NDB	NDB
BD1326-1	9.00a	9.00a	NDB
BD1308-3	6.00c	6.00c	7.00bc
BD1335-4	8.00ab	9.00a	NDB
BD1301-3	6.00c	6.00c	8.00ab
BD1330-3	8.00ab	9.00a	9.00a
BD1311-4	6.00c	8.00ab	7.00bc
BD1315-3	9.00a	8.00ab	NDB
BD1341-5	7.00bc	7.00bc	7.00bc
BD1421-5	8.00ab	8.00ab	7.00bc
BD1312-5	NDB	NDB	NDB

Treatment means followed by the same letter are not significantly different. NDB = No dormancy break.

3.2.2. Sprouting (%)

The main effects of locations and genotypes had significant effect on sprouting percentage of potato tubers and also had their interaction. Sprouting percentage (%) for genotypes and locations is presented in **Table 3**. The tubers harvested from Maine and Beltsville had highest sprouting percentage compared to the North Carolina harvested tubers.

The lowest sprouting percentage was noted in genotype BD1306-4 harvested from the Beltsville, while the highest percentage was noted in BD1308-3, BD1301-3 and BD1330-3 harvested from the Maine, and BD1308-3 and BD1341-5 harvested from the Beltsville (**Table 5**).

Table 5. Mean comparison of sprouting %age of tubers under the interactive effect of location × genotype.

Genotype	Location		
	Maine	Beltsville	North Carolina
BD1310-1	88.40fghi	93.81cde	82.83jkl
BD1334-2	45.17p	40.81p	43.35p
BD1306-4	34.76q	40.81r	33.77q
BD1326-1	82.74jkl	81.44l	73.55m
BD1308-3	100.00a	96.96abc	83.79jkl
BD1335-4	86.58ghij	85.70ijkl	57.63o
BD1301-3	100.00a	100.00a	100.00a
BD1330-3	100.00a	90.29efgh	81.44l
BD1311-4	92.11def	92.08def	82.96jkl
BD1315-3	81.59kl	88.35fghi	28.98r
BD1341-5	90.54efg	99.22ab	95.48bcd
BD1421-5	92.61cdef	88.91fghi	85.94hijk
BD1312-5	64.19n	71.05m	66.26n

Treatment means followed by the same letter are not significantly different.

4. Discussion

The variance among progenies for seedling vigor index (SVI) was significant at 5% level for all individuals. Parental tuber dormancy ranged from 6-10+ weeks and SVI ranged from 0 to 53.6. Correlation matrix is multivariate analysis which is helpful to determine direct and indirect association among different traits (Anjum et al., 2018; Ahmad et al., 2019). However, no correlation ($r = 0.01$) was observed between parental tuber dormancy and SVI. So, the relationship between offspring TPS dormancy and their parent's tuber dormancy is unpredictable[22-24].

The average temperature in Presque Isle, Maine ranges from 14-18°C, in Plymouth, North Carolina from 12-26°C and 18-24°C in greenhouse of BARC, Maryland during the potato growth period. So, the uplands experience consistently lower air and soil temperature than the lowlands. As shown in **Tables 4 and 5**, dormancy duration and sprouting percentage varied significantly under the interactive effect of location × genotype. The results of the current study are contradictory to many previous reports which found the minor impact of seed production site on dormancy duration and sprout growth (Wurr, 1979). The previous reports show a general agreement that temperature is the dominant factor in described effects of site of seed production on regrowth of progeny tubers. But, O'Brien and Allen (1986) rejected this interpretation of consistent relationship between temperature at the sites and dormancy duration. Their study suggested stage of crop growth at the time of defoliation and removal of tubers as determinants for dormancy duration[25-28].

One of the significant advantage of manipulating at the diploid level is that it is possible to breed for and fix traits under recessive genetic control, while it is almost impossible to fix recessive alleles at the tetraploid level. True potato seed of *Solanum tuberosum* is commonly used for preservation of wild germplasm and cultivated potatoes (Hawkes et al., 2000). TPS can be rapidly increased for agronomic and storage trials when a potential new hybrid cultivar is identified. In addition, while it is difficult to overcome dormancy in tubers. TPS dormancy can be easily overcome using gibberellic acid (Jansky et al., 2012). Simmonds (1963) suggested that selection of genotypes at seed level has

fundamental importance. The breeder can select for early sprouting by picking the first germinator in a seed lot and for slow sprouting (good keeping quality) could advance his selection by picking the slowest germinator. As potatoes are propagated through tubers, the increase of clonal material proceeds slowly (Haynes et al., 2012). For example, in ARS Potato Breeding Program, a genotype advances from a single hill in the first field generation to 60 hills in the third field generation. Genotypes in third field generation are available to cooperators for multi-location evaluation. By that time, less than 1% of the genotypes from the original population remain. Most potato breeding programs work in a similar fashion. In this study, the values of seedling vigor index of all the progenies having a long dormant parent, were lower except WHD-7, which had the high value of seedling vigor index with both parents were long-dormant (**Table 6**). Both parents of WHD-7 did not break their dormancy. WHD-2 and WHD-8 also had long dormant parents but had lower values (6.89 and 31.50) of seedling vigor index. The highest vigor index was observed in seedlings of WHD-4 which had both of its parents short-dormant [29-31]t.

Table 6. Seed and tuber dormancy in edible diploid potatoes.

Progenies	Parents (Female × male)	Parental tuber dormancy (weeks)	Seedling vigor index
WHD-1	BD1301-3 × BD1315-3	6.67, 9.00	3.10f
WHD-2	BD1306-4 × BD1335-4	NDB, 9.00	6.89ef
WHD-3	BD1306-4 × BD1341-5	NDB, 7.00	6.76ef
WHD-4	BD1308-3 × BD1330-3	6.33, 7.00	53.56a
WHD-5	BD1311-4 × BD1310-1	7.00, 9.00	11.94de
WHD-6	BD1311-4 × BD1334-2	7.00, NDB	0.00f
WHD-7	BD1312-5 × BD1334-2	NDB, NDB	49.05a
WHD-8	BD1315-3 × BD1310-1	9.00, 9.00	31.50c
WHD-9	BD1330-3 × BD1308-3	7.00, 6.33	40.97b
WHD-10	BD1341-5 × BD1301-3	7.00, 9.00	11.62de
WHD-11	BD1421-5 × BD1312-5	7.67, NDB	16.11d
WHD-12	BD1421-5 × BD1335-4	7.67, 9.00	3.85f
LSD G ($P \leq 0.05$)	10.367	7.564	

Treatment means followed by the same letter are not significantly different. LSD = Least significant difference.

5. Conclusion

From the results, it is clear that the relationship between offspring TPS dormancy and their parent's tuber dormancy is unpredictable. Additional research is needed to determine if selection for early sprouting in the offspring in vitro is correlated with short tuber dormancy in subsequent field generations of these offspring.

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