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BRIEF COMMUNICATION

Changes in abscisic and gibberellic acids contents during the release of potato seed dormancy

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Abstract

Gas chromatographic measurements demonstrated that the content of endogenous gibberellic acid increased and that of abscisic acid decreased during storage of potato seeds, suggesting that the dormancy of the seeds is controlled by the balance between these two hormones.

Additional key words: *Solanum tuberosum*.

Freshly harvested true potato seeds (TPS) have dormancy up to 6 months. Detailed investigations have been conducted on optimum conditions during germination of TPS. Gibberellins (GA_3) and charcoal accelerated speed of TPS germination (Lam 1968, Bamberg *et al.* 1986). Very little information is available about the physiological mechanism that controls the TPS dormancy. Spicer and Dinned (1961) demonstrated that dormant TPS may be induced to germinate by 24-h treatment with 2000 mg dm^{-3} GA_3. On the other hand, Simmonds (1963) found some inhibitory compound in the testa of TPS.

Since GA_3 and abscisic acid (ABA) have a role in control of seed dormancy in many species (*e.g.* Taylorson and Hendricks 1977), it was supposed that in TPS they might also have a role in dormancy. Therefore, in the present study, an attempt has been made to determine the changes in endogenous levels of ABA and GA_3 in TPS during dormancy.

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Abbreviations: DAH - days after harvest; DAS - days after sowing; ESTD - external standard; TPS - true potato seed.

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True seeds of potato (*Solanum tuberosum* L.) used in the present study were collected from the field-grown open pollinated plants of cv. Kufri Jyoti. Mature berries were collected and seeds extracted after storing the berries for 15 d. The seeds were air-dried and stored at room temperature.

Seven dormancy breaking treatments were tested (Table 1) with freshly harvested seeds. For scarification, the seeds were lightly rubbed between the folds of sand paper. Seed germination was recorded at day 20 after sowing.

Table 1. Comparison of various methods for release of TPS dormancy. Seeds were germinated on wet filter paper at 20 ± 1 °C in darkness for 20 d.

Treatment	Germination [%]
Untreated control	9.5
Scarification	37.0
Acetone dip. (10 min)	7.5
Sulphuric acid (2 min)	9.0
Hot water treatment (40 °C, 15 min)	6.0
Hot water treatment (40 °C, 30 min)	15.0
Prechilling treatment (7 d)	12.5
GA_3 2000 mg dm^{-3} (24 h)	71.5

The results revealed that at day 20 after sowing (DAS) maximum germination (71.5 %) was found after the GA_3, (2000 mg dm^{-3} for 24 h) treatment, followed by scarification (37.0 %). The percentage of germination was very low in all other treatments (Table 1).

For determination of presence of germination inhibitor 200 mg of dormant TPS were extracted 2 times with 50 cm^3 methanol for 1 h at room temperature and filtered. The methanol fractions were pooled and reduced at 40 °C to 50 cm^3, which are divided into 2 equal portions. One was stored as such and the second portion was further reduced to 10 cm^3 to which 20 cm^3 of phosphate buffer (0.02 M) pH 8.0 was added, slurried with insoluble polyvinylpyrrolidone (2 g) and filtered. After adjusting the pH of the filtrate to 2.5, with 10 % HCl, it was partitioned 3 times against 50 cm^3 ethyl acetate. The ethyl acetate fractions were pooled and reduced to 25 cm^3. 5 cm^3 of each extract was then layered on Petri dishes lined with filter paper. The extract was air-dried and 50 non-dormant weeds were put on each Petri dish along with 5 cm^3 of distilled water. The control consisted of distilled water only. Each treatment was replicated 3 times, the dishes were kept at about 20 °C in the dark and per cent germination recorded at 10, 15, 20 and 25 DAS.

Both methanol and ethyl acetate fractions reduced germination (Table 2) demonstrating that at 25 DAS some compound(s) is(are) present in the dormant TPS which inhibit(s) the germination of non-dormant TPS.

The possible presence of inhibitory substance in the testa of TPS was suggested by Simmonds (1963) but no attempt has been made to isolate and characterise this inhibitor. Our results show that the inhibitor was partitioned into the acidic ethyl acetate fraction. Therefore, we subjected the ethyl acetate fraction to gas

chromatographic analysis for the presence of ABA, an inhibitor of germination found in the seeds of many species (Taylorson and Hendricks 1977).

Table 2. Percentage seed germination in methanol and ethyl acetate fractions extracted from dormant seeds. Seeds were germinated in 5 cm^3 of the respective fractions.

Time after sowing [d]	Water control	Methanol fraction	Ethyl acetate fraction
10	30.0	20.0	22.0
15	43.0	29.2	31.2
20	68.0	43.0	34.6
25	75.4	48.6	41.3

Samples (200 mg of TPS stored at room temperature) were taken at 60, 90, 105, 120, 135, 150 and 165 d after harvest (DAH) of the berries and was fixed in methanol. Simultaneously, 50 seeds were tested for germination percentage. The method of Wurr *et al.* (1980) was adopted for the extraction and purification of GA_3 and ABA in seeds. Their quantification was performed on a gas chromatograph (*Model No. 5840A, Hewlett Packard,* USA) with flame ionization detector: *a*) ABA - the ethyl acetate fraction (equivalent to 100 mg TPS) obtained after purification was dried and derivatised with N,*o*-bis-(trimethylsilyl)-acetamide for 20 min at 27 °C. An aliquot equivalent to 20 mg TPS was then injected on the column packed with *UCW 982*. The column was maintained at 60 °C for 6 min and then the temperature increased up to 220 °C at rate 12.8 °C min^{-1}. The nitrogen flow rate was 23 cm^3 min^{-1} and injection port and detector temperature were maintained at 190 °C. The quantification was done by external standard method (ESTD). *b*) GA_3 - the ethyl fraction (equivalent to 100 mg TPS) was dried and derivatised with mixture containing pyridine and bis-(trimethylsilyl)-trifluoroacetamide in the ratio of 1:1 for 60 min at 27 °C. An aliquot equivalent to 20 mg TPS was injected into the column packed with *UCW 982*. The column was maintained at 180 °C for 5 min and then heated to 240 °C at the rate of 4 °C min^{-1}. The N_2 flow rate was 25 cm^3 min^{-1} and injection port and detector were maintained at 190 °C. The quantification was done by ESTD method using GA_3, as the GA_3 had a retention time of 22 min which was quite different from the retention times of other gibberellins tested (GA_4 and GA_7).

The germination was only 11.34 % by 60 DAH, thereafter reaching a maximum of 90 % by 150 DAH (Fig. 1). GA_3 content of TPS was low [17.9 μg g^{-1}(seed)] at 60 DAH. This gradually increased to 62.4 μg g^{-1}(seed) by 120 DAH by which time about 85 % seeds had germinated. Thereafter, the GA_3 contents declined (Fig. 1). ABA content of seeds, at 60 DAH was 9.3 μg g^{-1}(TPS), and gradually diminished and was as low as 2.1 μg g^{-1}(seed) at 135 DAH.

Thus, the dormant seeds had a high level of ABA which slowly decreased with time. Such a decrease in endogenous levels of ABA with release of dormancy has been demonstrated in other seeds *e.g.* of *Acer* (Webb and Wareing 1972, Petrova and Nikolaeva 1974). Thus, it can be argued that the dormancy of TPS is due to the presence of an inhibitory substance (ABA) which declines with storage.

Contrasting results are available regarding the role of gibberellins (GAs) in the dormancy release of TPS. One school postulates that GAs have a role in dormancy

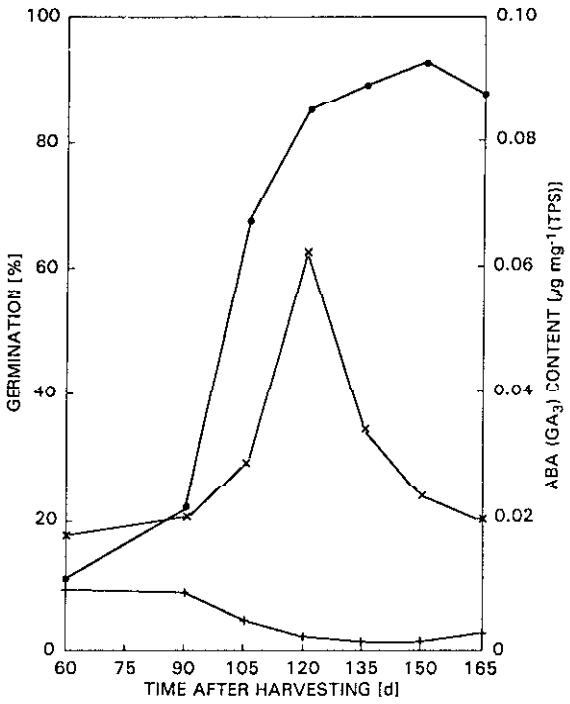

Fig. 1. Germination percentage (*closed circles*) and ABA (+) and GA_3 (×) contents at different intervals of storage of TPS.

release of TPS (Spicer and Dionne 1961, Lam and Erickson 1966, Lam 1968), while the second doubts their role (D'Antonio and McHale 1988, Pallais 1989), because they got lower percentage in GA-treated dormant seeds as compared to non-dormant seeds. Moreover, the seedling produced from GA-treated dormant seeds had less dry matter as compared to non-dormant seeds. In the present study, the GA_3 application was found to be the best treatment for dormancy release. Also, there was a gradual increase in the endogenous levels of GA_3, with the increase in germination percentage. Thus, our results support the first hypothesis. Moreover, in the present study it has been demonstrated that the endogenous levels of an inhibitor (ABA) declines whereas those of a promoter (GA_3) increases with dormancy release. Therefore, it is postulated that the dormancy of TPS is controlled by the promoter-inhibitor balance as has been suggested by Khan (1975) for other crops.

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