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Methods for breaking dormancy and germination of tuberose (*Polianthes tuberosa*) seeds

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ABSTRACT : The seed germination study is the utmost important character to develop new hybrids after successful fruitset. But the seeds of tuberose did not show much response under favourable climatic condition. Hence, the aim of the study was to break the dormancy and increase its germination by using various chemicals *viz.*, gibberellic acid, thiourea, potassium nitrate and indole butyric acid. Seed treatment with GA_3 @ 250 ppm for 8 hrs was found to be effective in improving the germination by 12.50 per cent which was 63.68 per cent higher than control.

KEY WORDS : Tuberose, Seeds, Germination

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Tuberose (*Polianthes tuberosa* Linn.) is an important commercial flower crop in India and is popular due to its fragrance and long keeping quality of flower spikes (Sadhu and Bose, 1973). The spikes are useful as cut flowers for vase decoration (Benschop, 1993) and bouquets while individual flowers are used for making veni, garland and buttonholes. It is believed to have originated in Mexico (Bailey, 1903). It belongs to the family Amaryllidaceae (Bailey, 1939). The seeds of tuberose are black coloured, half circular and 3 mm in diameter. Normal germination test for assessment of vaiability of the seed sample exhibited no germination, indicating 100 per cent seed dormancy due to hard-seedness. The major hurdle in crossing of tuberose genotypes is that fruit setting and seed setting is arrested in many crosses. Among the crosses made, only few crosses could produce few seeds, but later these seeds failed to germinate (Krishnamurthy, 2000). Hence, assessing seed germination in the genotypes is very much essential. No information is available on seed germination and dormancy breaking regimes of this genus. Hence, recognising its importance and there is a need to develop a method for breaking seed dormancy. Therefore, various methods for breaking the hard seed coat dormancy were attempted.

Dormancy is a condition in which seeds do not germinate even when the environmental conditions (water, temperature and aeration) are permissive for germination (Nikolaeva, 1977; Bewley and Black, 1994 and Hartmann *et al.*, 1997). Various methods have been used by seed scientists and technologists to break seed dormancy. The chemicals which are used commercially in various places are including : potassium nitrate, thiourea, sulphuric acid, ethanol and cynamid. All of these chemicals are inexpensive and can be used easily to break the true dormancy of seeds effectively (Chang and Sung, 2000). Many investigators have studied the effects of exogenous growth regulators on seed germination. Potassium nitrate showed improvement in the seed germination of African marigold due to formation of nitarte during imbibitions. It would have provided additional substrate for aminoacid and protein synthesis for the enhancement of germination (Selvaraju and Selvaraj, 1994). Among other chemicals, potassium nitrate and thiourea are widely used to break dormancy (Agrawal and Dadlani, 1995). However, thiourea overcomes certain types of dormancy, such as the seed-coat inhibiting effect of deep embryo-dormant *Prunus* seeds (Hartmann *et al.*, 1997). To accelerate breaking seed dormancy, hormones have been applied in several studies

(Zigas and Coombe, 1977; Mehanna *et al*., 1985 and Chang and Sung, 2000). Gibberellic acid (GA_3) is one of the hormones proposed to control primary dormancy by inducing germination (Iglesias and Babiano, 1997). It eliminated the chilling requirements of peach and apple seeds and increased their germination (Rouskas *et al.*, 1980 and Mehanna *et al.*, 1985). Lack of literatures on comprehensive study of tuberose seed dormancy breaking is still obvious. Therefore, the objectives of this study were to assess the effects of different seed dormancy breaking treatments on seed germination and devise an effective method for breaking seed dormancy of tuberose.

RESEARCH METHODS

This research was carried in faculty of Horticulture, Tamil Nadu Agricultural University, Coimbatore. Mature open pollinated seeds of tuberose were collected from the germplasm maintained in Botanical Garden, Tamil Nadu Agricultural University in the month of September, 2012 and immature and damaged seeds were removed and dry seeds were used for this experiment. In order to overcome the hard-seededness, the seeds were subjected to the following treatments to break the physical dormancy imposed by hard seed coat. Untreated seeds were used as control.

Soaking treatment :

Seeds were soaked in water for 8 and 16 hours.

Chemical treatments :

Gibberellic acid :

Gibberellic acid was applied in two concentrations (250 and 500 ppm) for 8 and 16 hours.

Potassium nitrate :

Potassium nitrate was applied in two concentrations (0.25 and 0.5 %) for 8 and 16 hours.

Thiourea :

Thiourea was applied in two concentrations (0.5 and 1.0 %) for 8 and 16 hours.

Indole butyric acid :

Indole butyric acid was applied in two concentrations (100 and 200 ppm) for 8 and 16 hours.

Measured traits :

Germination percentage, root length, shoot length, vigour index, dry matter production and seed viability.

Germination percentage :

Four replicates of 100 seeds each were germinated in pots filled with sand (ISTA, 1993) and kept in the germination room maintained at 25 ± 2°C temperature and 90 ± 5 per cent RH. Count was made on the number of normal seedlings at 28 days after sowing and the germination was calculated and expressed as percentage.

Root length :

The root length of the 10 normal seedlings was measured from the collar region to the tip of the primary root and expressed in cm.

Shoot length :

10 normal seedlings were selected at random and length of the shoot was measured from collar region to the growing tip. The mean shoot length was expressed in centimeter.

Vigour index :

The vigour index was calculated by using the formula suggested by Abdul- Baki and Anderson (1973) and expressed as whole number.

Vigour index= germination percentage×(root length + shoot length)

Dry matter production :

The 10 normal seedlings utilized for seedling measurements were first dried under shade and then dried in a hot air oven maintained at 85 ± 1°C for 24 hours. After drying, they were cooled in a desiccator for 30 minutes, weighed and expressed in gram.

Statistical analysis :

The data were statistically analyzed using a Completely Randomized Design with three replications. Data were subjected to analysis of variance using the AGRESS software package.

RESEARCH FINDINGS AND DISCUSSION

The seed germination study of tuberose did not show much response under favourable climatic condition. The present study revealed that the seed treatment with GA_3 @ 250 ppm for 8 h^{rs} improved the germination by 12.50 per cent which is 63.68 per cent compared to control (Table 1). The increase in germination due to GA_3 treatment was claimed as due to breakdown of starch and other substrates that induced the enzyme action, the first step of the germination process which created an ability to overcome a metabolic block in the embryonic axis of endosperm (Cneudt and Boseman, 1983) and also they demonstrated that low concentration of GA_3 could stimulate amylase production in the absence of germination. But Jouret (1977) reported that GA_3 did not influence the synthesis of total RNA in aleurone tissue. However, Heit (1971) concluded that GA_3 promoted the synthesis of specific mRNA responsible for *in vitro* synthesis of α amylase between 5-8 hrs of GA_3 treatment. Hence, this could be the reason for the enhanced germination

obtained in the present study with GA_3 compared to other growth regulators. Thompson (1970) also reported that GA_3 enhanced the activity of endo-β-1, 4-xylanase in the aleurone layer. The multiplicity of the effects of GA_3 in the regulation of enzyme synthesis and secretion in aleurone cells indicates that this hormone has the potential to regulate germination in numerous ways.

Wilfret and Green (1976) also demonstrated that GA_3 could enter into chemical combination with phospholipids (Wlamer *et al*., 1974) and, thus, increased the germination. In the present study, when GA_3 was used at higher concentration, it decreased the germination significantly due to the lethal activity stimulated on enzyme reaction at supra optimal condition as reported by Natarajan (2003) in petunia. Thus, the study indicated that optimum concentration of GA_3 for enhancing the seed germination of tuberose was 250 ppm. The other characters evaluated on seedling quality *viz.*, root length (13.24 cm), shoot length (14.32 cm), drymatter production (0.24 g) and vigour index values (978.38) were also higher in seeds treated with GA_3 250 ppm for 8 hours which was attributed to the increase in cell division and proliferation to root and apical meristem tissues by Lal *et al.* (1971) and Das *et al*. (1999).

Next to the treatment involving GA_3, seeds treated with KNO_3 0.5 per cent for 8 hours resulted higher germination percentage and also in seedling quality *viz.*, root length (13.00 cm), shoot length (14.23 cm), drymatter production (0.22 g) and vigour index values (965.30) (Table 2). The improvement in the seed germination may be attributed to the presence of nitrate formed during imbibitions. It would have provided additional substrate for aminoacid and protein synthesis for the enhancement of germination. Such promotary effects of potassium nitrate have also been reported in African marigold (Selvaraju and Selvaraj, 1994; Hadwani *et al*., 2013 and

Table 1 : Influence of pre sowing treatment with growth regulators on seed and seedling characteristics in tuberose

Growth regulators (G)	Germination (%)			Root length (cm)			Shoot length (cm)		
	Duration in hours (D)								
	8	16	Mean	8	16	Mean	8	16	Mean
GA_3 250 ppm	12.50	11.32	11.91	12.36	13.24	12.80	13.75	14.32	14.04
500 ppm	11.12	10.09	10.61	10.54	10.72	10.63	9.77	10.12	9.95
KNO_3 0.25 %	12.45	11.23	11.84	13.00	11.60	12.30	14.23	12.89	13.56
0.5 %	12.12	10.13	11.13	12.63	10.23	11.43	13.65	11.29	12.47
Thiourea 0.5%	09.21	09.05	9.13	10.53	9.82	10.18	9.98	9.29	9.64
1%	10.45	09.87	10.16	10.80	9.12	9.96	9.14	8.98	9.06
IBA 100 ppm	09.15	8.23	8.69	9.66	8.98	9.32	9.12	8.56	8.84
200 ppm	09.98	9.21	9.60	9.45	9.12	9.29	8.78	8.50	8.64
Water soaking	7.96	7.96	7.96	7.46	7.46	7.46	7.21	7.21	7.21
Mean	11.23	10.76	10.96	9.12	8.97	9.05	8.43	8.21	8.32
	G	D	GD	G	D	GD	G	D	GD
C.D. (P=0.05)	2.17	2.00	3.08	0.134	NS	NS	NS	NS	NS

NS= Non-significant

Table 2 : Influence of pre sowing treatment with growth regulators on vigour index and dry matter production of tuberose seedling

Growth regulators (G)	Vigour index			Dry matter production (g/seedling^{-5})		
	Duration in hours (D)					
	8	16	Mean	8	16	Mean
GA_3 250 ppm	892.70	978.38	935.54	0.23	0.24	0.24
500 ppm	591.43	606.24	598.88	0.18	0.19	0.19
KNO_3 0.25 %	965.30	740.33	852.82	0.22	0.20	0.21
0.5 %	843.59	725.34	784.47	0.21	0.18	0.20
Thiourea 0.5 %	496.55	440.49	468.52	0.17	0.15	0.16
1 per cent	467.59	413.95	440.77	0.15	0.13	0.14
IBA 100 ppm	434.76	412.24	423.50	0.12	0.11	0.12
200 ppm	418.93	415.32	417.12	0.14	0.13	0.14
Water soaking	263.47	263.47	263.473	0.04	0.04	0.04
Mean	795.87	765.54	780.71	0.03	0.03	0.03
	G	D	GD	G	D	GD
C.D. (P=0.05)	0.261	NS	NS	0.217	NS	NS

NS = Non significance

Singh and Singh, 2013).

Conclusion :

In conclusion, the results suggest that since GA_3 and KNO_3 both induced seed germination of tuberose but the success percentage was minimum. Therfore, the ability of seed germination in this plant is a complicated process that is controlled by both external and internal regulating factors. Since application of GA_3 and KNO_3 are not able to enhance germination to its maximum level but thay are helpful for the development of hybrids after sucessful crossing by accelerating its germination.

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