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Effect of growth regulatory hormones on the germination stored seeds of *Withania somnifera*

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Abstract : In the present study the effect of gibberellic acid (GA_3) and kinetin (KIN) on the germination and dormancy on the stored seeds of medical plant, *Withania somnifera* (Var. WS 20) has been studied. Seeds of *Withania somnifera* (Var. WS 20) were stored at constant temperature in desiccators and the germination data was taken up to 2 years with interval of 6 months. The parameters include are speed of germination (QI) , average root length (SVI) and average total length (SVI – II). In addition to these parameters, the measurements of average root length, average shoot length, average total length, average fresh weight, and average dry weight, average root length, average shoot length, average total length, average fresh weight, average dry weight were also calculated. The quantity/concentration of both plant growth regulators was used between 10^{-5}M- 10^{-7} M. The presoaking treatment was given to seeds. The findings of the study show that gibberellic acid (GA_3) with 10^{-5} M and kinetin (KIN) 10^{-7}M are better for acquiring % germination (%G), the quality index and seedling vigour index (SVI-1 and SVI-11) in fresh and partially aged (1 year and 2 year) seeds . Comparative data suggest that the storage potential of seeds, *Withania somnifera* (Var. WS 20) can be improved significantly with the help of PGRs.

Keywords : Regulatory hormones, Germination, *Withania somnifera*

Introduction

The term plant hormone for growth regulating substances was introduced by Fitting (1909). The seed is one of the astonishing innovations of nature and it is now established that plant hormones affect seed germination by acting on different parts of the seed (Srivastava, 2002). Recent advances in agricultural research has suggested that the improvement in crop productivity and quality can be further improved by incorporating new technologies into traditional breeding programs and influence harvesting stages on seed vigour (Anonymous, 1985 & Mhatre and Rao, 1998). Grove *et al*. (1979) reported that brassinolide is a plant growth-promoting steroid, which they isolated from *Brassica napus* pollen. Tsai F-Y *et al*. (1997) made a comparative study of the effects of abscisic acid and methyl jasmonate, plant growth regulators, on seedling growth of rice. They found that growth regulators promote flowering, cellular division, and in seeds growth after germination. Gopikumar and Moktan (1994) studies the effects of plant hormones on seed germination and growth of true seedlings in the nursery. Tsai *et al*. (1997) studied the metabolism of gibberellins and suggested that gibberellins are important in seed germination affecting enzyme production that mobilizes food production used for growth of new cells. Saxena (1974) described the presowing hardening treatments improves crop production. Ethylene upregulate auxin biosynthesis in *Arabidopsis* seedlings to inhibition of root cell elongation (Swarup et al, 2007). Nitric oxide signaling in plants and help in the synthesis in the chloroplast (Shapiro, 2005 & Roszer, 2012). Keeping in view the aforesaid facts a study was taken to observe the seedling vigour of fresh and partially aged seeds (6 month to 2 year old seeds and to improve the seedling vigour with presoaking treatment of Gibberellic Acid (GA_3) and Kinetin (KIN) on germination.

Methodology

Studies of germination are divided into different steps during 2005 as per the procedure followed by Agrawal and Dadlani (1987).

Plant Growth Regulators (PGRs) were procured from Sigma laboratory. The Seeds were treated with PGRs for experimental purpose. The seeds were dried and soaked in different concentrations of gibberellic acid (GA_3) ranging between 10^{-5}M to 10^{-7}M. Similarly the same concentration was taken for kinetin (KIN) in distilled water. Soaking period of seeds was for 5 hours. Then seeds were removed from the solution and transferred to the tray, which was covered with filter paper and dried at room temperature till than original weight was measured. Dry seeds were put for germination up to the final count.

Hormones preparation

Stock solution of GA_3 (10^{-3}M): Accurately weighed 34.64 mg GA_3 was dissolved in 2 ml acetone and final volume was made up to 100 ml DW. Different concentrations (10^{-5}M to 10^{-7}M) were prepared from stock.

Stock solution of KIN (10^{-3}M): Accurately weighed 21.52 mg KIN was dissolved in 2 ml 1N NaOH and final volume was made up to 100 ml DW.

Seeds were germinated in sterilized chamber. Intensity of light was maintained at 500 lux from tube light. e: Constant temperature 25 ± 2^0C was maintained during germination, the relative humidity of the room was kept at 50-55%.

Germination parameters were recorded as suggested by ISTA (1985 a,b):

Normal seedling count, abnormal seedling count were counted. Germination percentage (%G) was calculated as follows on the basis of the number of normal seedlings as suggested by Agrawal (1987).

$$\%G = N / T \times 100,$$

N = Number of normal seedlings, T = Total number of seeds kept for the germination

Seedling length was recorded in cm and divided into root length and shoot length.

Fresh and dry weight of seedlings: Average fresh weight (per seedling) was recorded. Normal seedlings were packed in blotting paper and kept in oven at 80 ± 2^0C till constant dry weight was recorded.

The formula quality Index (QI) or Speed of germination was calculated as follows (Maguire, 1962);

$$QI = \frac{\text{Number of seeds germinated}}{\text{Day of inspection}}$$

Seedling Vigour Index (SVI): SVI-I and SVI-II were calculated according to method given by Jayraj and Karivartha Raju (1992) and Abdul Baki, James and Anderson (1973): The formulae are as follows:

SVI-I = %G X Dry weight of seedling

SVI-II = %G X Total length of seedling

Results and Discussion

Plant hormones are considered effective molecule in development of seeds. The hormones affect seed germination and dormancy by acting on different parts of the seed. Walz *et al*. (2002) stated that there is a correlation of auxins and cytokinins in plant, known as a A/C= constant. They further held that a gene encoding a protein modify by the phytohormones, indoleacetic acid acting by modulating chromosomal transcription. Gibberellins include a large range of chemicals that are produced naturally within plants and are important in seed germination, affecting enzyme production that mobilizes food production used for growth of new cells (Agrawal and Dadlani,1987). Gopikumar and Moktan (1994) studies on the effects of plant hormones on seed germination and growth of true seedlings in the nursery and found that plant hormones are suitable to cover the dormancy due to storing of seeds and initiate germination**.** Plant hormones like GA_3 and KIN act upon a responsive plant system by interaction the molecules and effect the morphological, physiological and biochemical responses. Chinoy (1942, 1967) had first time tried presowing treatment of PGRs in wheat seeds. The critical studies were made on proper concentration, soaking volume and application of PGRs on Indian plants (Saxena, 1974, 1990; Murlikrishna, 1993). In the present investigation the pretreated stored seeds were with 10^{-5} M to 10^{-7} M of GA_3 and KIN kept in distilled water for five hours gave good (Table 2 & Table 3). The results were better in comparison to control (Table 1). The present study is in conformity with the observations of earlier investigation in the field (Chinoy, 1942 & 1967; Saxena, 1974 & 1990; Murlikrishna, 1993). Out of three concentration of GA_3 and Kin (10^{-5}M, 10^{-6}M, 10^{-7}M), GA_3 (10^{-5} M) gave more germination percentage (%G, 60), seedling length (3.3), fresh and dry weight, quality index (QI, 2.6), Seedling Vigour Index (SVI-1,40.20) and(SV1-II,330.0) are more as compare to control. On the other hand, KIN (10^{-7} M) is better results in r fresh and partial (1 year and 2 year) aged seeds in % G , Q.I., SVI-I and SVI-II. The PGRs are known to break seed dormancy in a number of plants (Gopikumar and Moktan, 1994).

Table 2: GA_3 treatment to Fresh and Partially aged seeds of WS 100 variety of *Withania somnifera*

Treatments	No. of seeds	Normal seedlings	Abnormal seedlings	% G	Average root length (cm)	Average shoot length (cm)	Average total length (cm)	Average fresh weight (mg)	Average dry weight (mg)	Q.I.	SVI - I	SVI - II
Treatment to 0 Month Old seeds (Fresh seeds)												
$GA_3 10^{-5}$M	25	15	0	60	3.3	2.2	5.5	34.60	0.67	2.67	40.20	330.0
$GA_3 10^{-6}$M	25	15	0	60	2.0	1.5	3.5	27.33	0.40	2.67	40.20	210.0
$GA_3 10^{-7}$M	25	12	3	48	1.2	1.2	2.4	25.90	0.36	2.12	17.28	115.2
Treatment to 12 Month Old seeds												
$GA_3 10^{-5}$M	25	12	0	48	1.4	1.2	2.6	25.00	0.62	1.85	29.76	124.8
$GA_3 10^{-6}$M	25	9	0	36	1.2	1.5	2.7	27.77	0.55	1.28	19.80	97.2
$GA_3 10^{-7}$M	25	6	0	24	1.0	1.5	2.5	42.50	0.48	0.86	11.52	60.0
Treatment to 24 Month Old seeds												
$GA_3 10^{-5}$M	25	5	1	20	3.0	1.0	4.0	52.00	0.70	0.20	14.00	80.0
$GA_3 10^{-6}$M	25	6	4	24	1.8	1.2	3.0	40.00	0.42	1.45	10.08	72.0
$GA_3 10^{-7}$M	25	6	0	24	1.0	1.2	2.2	28.33	0.52	0.69	12.48	52.8

Table 3: KIN treatment to Fresh and Partially aged seeds of WS 100 variety of *Withania somnifera*

Treatments	No. of seeds	Normal seedlings	Abnormal seedlings	% G	Average root length (cm)	Average shoot length (cm)	Average total length (cm)	Average fresh weight (mg)	Average dry weight (mg)	Q.I.	SVI - I	SVI - II
Treatment to 0 Month Old seeds (Fresh seeds)												
KIN 10^{-5} M	25	8	3	32	2.6	1.9	4.5	36.9	0.50	1.01	16.00	144.0
KIN 10^{-6} M	25	9	3	36	3.3	2.3	5.6	31.7	0.33	1.54	11.88	201.6
KIN 10^{-7} M	25	14	1	56	1.2	2.2	3.4	33.6	0.38	2.21	21.28	190.4
Treatment to 12 Month Old seeds												
KIN 10^{-5} M	25	6	4	24	1.8	1.3	3.1	40.0	0.43	1.40	10.32	74.4
KIN 10^{-6} M	25	5	1	20	3.0	1.1	4.1	52.0	0.71	0.23	14.20	82.0
KIN 10^{-7} M	25	12	3	48	1.2	1.0	2.2	25.9	0.36	2.12	17.28	105.6
Treatment to 24 Month Old seeds												
KIN 10^{-5} M	25	5	0	20	0.8	1.0	1.8	27.2	0.21	0.20	4.20	36.0
KIN 10^{-6} M	25	6	2	24	0.7	1.1	1.8	28.9	0.25	0.38	6.00	43.2
KIN 10^{-7} M	25	7	0	28	0.9	1.6	2.5	35.0	0.40	0.52	11.20	70.0

Table1: Seed vigour and germination data of WS 100 variety of *Withania somnifera*

Storage period	No. of seeds	Normal seedlings	Abnormal seedlings	% G	Average root length (cm)	Average shoot length (cm)	Average total length (cm)	Average fresh weight (mg)	Average dry weight (mg)	Q.I.	SVI - I	SVI - II
0 Month	25	14	0	56	1.5	1.0	2.5	21.73	0.65	1.35	36.40	148.0
6 Month	25	14	0	56	1.0	1.0	2.0	21.73	0.50	1.83	25.00	112.0
12 Month	25	14	0	56	0.7	1.2	1.9	21.42	0.39	1.54	21.84	106.4
18 Month	25	10	0	40	0.9	1.0	1.9	29.00	0.45	1.23	18.00	76.0
24 Month	25	9	0	36	0.9	1.0	1.9	29.44	0.49	0.93	17.64	68.4

Referance

Abdul Baki A. A., James and Anderson (1973): Vigour estimation in soyabean by multiple criteria, *Crop Sci.,* **13:** 630-633

Agrawal P. K. (1987): Germination test under controlled conditions and its evaluation, In: *Techniques in seed science and technology.* (Eds.) Agrawal, P. K. and Dadlani, M. (South Asian Publishers, New Delhi)

Agrawal P. K. and Dadlani, M. (Eds.) (1987): *Techniques in seed, science and technology*, (second edition, South Asian publishers, New Delhi)

Anonymous (1985): International rules for seed testing, *Seed Sci. & Technol.,* 13 (2): 307-520

Chinoy J. J. (1942): Presowing treatment and phasic development, *Curr.Sci.,* **11,** 400

Chinoy J. J. (1967): Role of ascorbic acid in crop production, *Poona Agr. College Magaz.*, **57:** 1-6

Fitting H. (1909): Die Bee influssung der orchideenbluten durch die bestaubung und durch andere umstande, *Z. of Bot.,* **1:** 1

Gopikumar K. and Moktan M. R. (1994)**:** Studies on the effects of plant hormones on seed germination and growth of true seedlings in the nursery, *J. Tropical Forestry,* **10:** 45-50

Grove M. D.; Spencer G. F.; Rohwedder W. K.; Mandava N.; Worley, J. F.; Warthen J. D.; Steffens G. L.; Flippen-Anderson J. L. et al. (1979): Brassinolide, a plant growth-promoting steroid isolated from Brassica napus pollen. *Nature.* **281,** 5728

International Seed Testing Association (ISTA) (1985 **a)**: International rules for seed testing, rules 1985, *Seed Sci. & Technol.,* **13:** 299-355

International Seed Testing Association (ISTA) (1985 b): International rules for seed testing, annexes 1985, *Seed Sci. & Technol.,* **13:** 358-513

Jayraj T. and Karivartha Raju T. V. (1992): Influence of harvesting stage on seed vigour in groundnut cultivars, *Seed Research*, **20:** 41-44

Maguire J. D. (1962): Speed of germination - a new vigour test for crop seeds, In: *Seed production.* (Ed.) Habblethwaite (Butterworth, London). pp, 647-660

Mhatre M. and Rao P. S. (1998): Plant tissue culture - current trends and future prospects, In: *Advances in plant physiology,* (Ed.) Hemantranjan A. (Sci. Publ., Jodhpur, India) pp: **72**-101

Murli Krishna S. (1993): Physiological studies on castor and cotton, Ph.D. Thesis, Gujarat University, Ahmedabad

Roszer T. (2012): Nitric Oxide Synthesis in the Chloroplast. In: The Biology of Subcellular Nitric Oxide (Ed) Roszer T. (Springer New York London)

Heidelberg).

Saxena O. P. (1974): Presowing hardening treatments and crop production – a review, *Planta,* **4:** 1-11

Saxena O. P. (1990): Some aspects of seed germination, In: International Symposium Environmental Mechanism. In Recant Advances in Research and Technology, Jodhpur. Jan. 27-28

Shapiro A. D. (2005): Nitric oxide signaling in plants. Vitam Horm. **72**:339-98.

Srivastava, L. M. (2002). *Plant growth and development: hormones and environment*. (Academic Press). p. 140.

Swarup R., Perry P., Hagenbeek D. (2007): Ethylene upregulates auxin biosynthesis in *Arabidopsis* seedlings to enhance inhibition of root cell elongation

Tsai F-Y.; Lin C.C.; Kao C.H. (1997): A comparative study of the effects of abscisic acid and methyl jasmonate on seedling growth of rice. *Plant Growth Regulation* **21** (1): 37–42.

Walz A, Park S, Slovin JP, Ludwig-Müller J, Momonoki Y. S, Cohen J. D (2002). "A gene encoding a protein modified by the phytohormone indoleacetic acid". *Proc. Natl. Acad. Sci. U.S.A.* **99** (3): 1718