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EFFECT OF PGRS WITH AND/OR WITHOUT RED LIGHT ON SEED GERMINATION OF ASHWAGANDHA (*WITHANIA SOMNIFERA* DUNAL) UNDER IN VIVO AND IN VITRO CONDITIONS

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ABSTRACT:

Ashwagandha (*Withania somnifera* Dunal) is one of the important medicinal and ethnobotanical plant. It is from Solanaceae family which has typical characteristic of seed dormancy due to hard seed coat. Its germination is not as easy as per commercial scale so that, the dormancy has to be broken with different PGRs treatment like GA_3 (10^{-5} M to 10^{-7} M) and KIN (10^{-5} M to 10^{-7} M) with / without red light under *in vivo* and *in vitro* condition. Presoaking treatment of PGRs with / without red light was given to seeds as a part of *in vivo* seed technology. Under the treatment of PGRs + red light, less % G but healthy plants produced. Hormonal condition of MS media with / without red light was used as a part of *in vitro* plant tissue culture technique. It was observed that the germination time period was reduced from 30 days to 5-7 days and healthy 10-15 multiple shoots and roots (which may lead whole plantlet in future) from a single seed in MS + 10mg/l KIN + red light. This technique may be effective in other examples of hard seed coated plants like Solanaceae.

Key Words: *Seed germination, seed dormancy, in vivo, in vitro, % G, multiple shoots and roots*

INTRODUCTION:

The seed is one of the astonishing innovations of nature, which encloses within itself a promise of life. Recent advances in agricultural research have indicated that improvement in crop productivity and quality can be better achieved by incorporating new technologies into traditional breeding programs (Mhatre and Rao, 1998). Moisture, temperature and oxygen are considered essential and it may be required for some kind of seeds. The emergence and development of the seedling to a stage where the presence, absence and formation of essential structure can be assessed thus indicating whether or not the seedling is able to develop further into a stationary plant under favorable condition in soil (Anonymous, 1985). This led to the development of the concept of seed vigour, whereby seedlot with high laboratory germination, which emerge poorly in the field are referred to as low vigour lots, while high

vigour lots emerge well. Many possible reasons for differences in vigour are genotype, seed size, seed density and the incidence of seed borne pathogens. Of the recently evaluated time emerging biotechnologies expected to make contribution in 21st century, plant growth regulators (PGRs) is one of them. The early use of the term hormone for growth regulating substances from plants was used by Fitting in his description on the effects of aqueous extract of orchid pollen on floral organs (Fitting, 1909). Plant tissue culture is the aseptic culture of plant cells, tissues and organs on a defined nutrient medium under controlled environmental condition to generate organized whole plants. In the context of plant tissue culture callus is a largely unorganized proliferating mass of parenchyma cells. Its induction required three equally important considerations (1) The selection of an explant (2) The provision of a suitable nutrient medium and culture conditions (3) The isolation and maintenance of callus for subsequent experimentations. The goal of it is to obtain quickly, a large number of genetically identical, physiologically uniform and developmentally normal plantlets, preferably with a high photosynthetic or photoautotrophic potential and survive the harsh *ex vitro* condition at a lowered cost.

Keeping in view the aforesaid facts an extensive study was taken up with the following broad objectives:

1. To examine the efficacy of red light treatment under *in vivo* and *in vitro* germination
2. To determine the effect of PGRs with and/or without red light under *in vivo* germination
3. To standardize the tissue culture media and examine the effect of PGRs with and/or without red light under *in vitro* germination

PLANT MATERIALS:

Scientific name : *Withania somnifera* Dunal

Family : Solanaceae

Variety : WS-20

METHODOLOGY:

(A) *IN VIVO* SEED GERMINATION: Studies of germination are divided into different steps (Agrawal and Dadlani, 1987);

(1) Requirements: These were as follows:

(1.1) Substratum: Germination filter paper was used for the method of 'Top of paper (TP)'. Seeds were germinated on top of paper in a petridish, which was covered with a lid to restrict moisture loss.

(1.2) Plant Growth Regulators (PGRs) or Hormones: Seeds were treated with PGRs and DW for better results.

- **Hormone soaking treatment:**

Dry seeds were soaked in different concentrations of gibberellic acid (GA_3), (10^{-5}M to 10^{-7}M), kinetin (KIN) (10^{-5}M to 10^{-7}M) and distilled water (DW). Amount of it was double then the seed weight (1 g seeds in 2 ml solution). Soaking period was for 5 hours. 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 obtained. Dry seeds were put for germination up to the final count. This period was 28-30 days for *Withania somnifera* seeds. The germination filter paper was changed frequently to prevent microbial growth.

- *Hormones preparation:*

(1) 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.

(2) 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. Different concentrations (10^{-5}M to 10^{-7}M) were prepared from stock.

(1.3) Germination Room (Growth chamber): Seeds were germinated in sterilized chamber or room in which temperature and light was adjustable.

(2) Physiological factors: Different factors played important role in germination.

(2.1) Light:

- Normal light: It was given continuous during germination period. Intensity of it was 500 lux from tube light.
- Red light: It was given continuous during germination period.

(2.2) Temperature: Temperature was constant during germination and data were recorded at 25 ± 2^0C.

(2.3) Humidity: Relative humidity of the room was 50-55%.

(3) Germination parameters: Different parameters were recorded as follows (ISTA, 1985 a,b):

(3.1) Normal seedling count: Seedling showed the continue development into satisfactory plants. Non-infected and intact seedlings were counted as normal.

(3.2) Abnormal seedling count: Damaged, deformed, unbalanced or decayed seedlings were counted as abnormal.

(3.3) Germination percentage (%G): It was calculated on the basis of the number of normal seedlings (Agrawal, 1987).

$$\%G = \frac{N}{} \times 100$$

T

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

(3.4) Seedling length: It was recorded in cm and divided into 2 subparts; Root length & Shoot length

(3.5) 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 $\pm$ 2^0C till constant dry weight was recorded.

(3.6) Quality Index (QI) or Speed of germination: The formula was given by Maguire (1962);

QI= Σ Number of seeds germinated

Day of inspection

(3.7) Seedling Vigour Index (SVI): It was calculated with that the help of two different formulas;

- SVI-I was given by Jayraj and Karivartha Raju (1992) as follows:

SVI-I = %G X Dry weight of seedling

- SVI-II was given by Abdul Baki, James and Anderson (1973) as follows:

SVI-II = %G X Total length of seedling

(B) *IN VITRO* SEED GERMINATION:

Seeds were taken as explants and inoculated on basal MS (Murashige and Skoog, 1962) or hormonal MS medium. It was germinated fast as compared to *in vivo* (in petridish) and developed into whole plantlets. Subculture was necessary for one-two times. Plantlets were transferred into field also.

RESULTS AND DISCUSSION:

- ***In vivo* seed germination:** Hormones are considered effective molecule in development of seeds. When different hormones like GA_3, KIN act upon a responsive plant system, it of course, enter in some direct interaction of molecules which are present in the seed which results eventually in manifestation of measurable effects in terms of morphological, physiological and biochemical responses. The mechanism by which minute amount of plant hormones, relatively simple organic compounds can do dramatically controlled growth is one of the challenging problems in plant physiology. The seeds were pretreated with 10^{-5} M to 10^{-7} M GA_3, 10^{-5} M to 10^{-7} M KIN and DW for five hours. From the germination one can observe that different concentrations of GA_3 (10^{-5}M, 10^{-6}M, 10^{-7}M), KIN (10^{-5}M, 10^{-6}M, 10^{-7}M) and DW soaking treatments gave more germination percentage (%G), seedling length,

fresh and dry weight, quality index (QI), Seedling Vigour Index (SVI) I and II as compare to control. Chinoy (1942, 1967) had first time tried presowing treatment of PGRs in wheat seeds. Proper and critical studies are needed on right PGRs, their concentration and proper application as well as soaking volume and duration (Saxena, 1974, 1990; Murlikrishna, 1993). The PGRs are known to break seed dormancy in a number of plants (Gopikumar and Moktan, 1994). Mehanna et al., 1985 suggested that GA_3 alter metabolism of seeds and trigger seed germination. Following are the output of results **(Table 1 & Table 2)**;

1. Untreated control seeds with red light produced less % G with healthy plantlets as compare to without red light.
2. Without red light : Treatment of GA_3 10^{-5} M is better than other all the given treatments.
3. With red light : No. of Abnormal seedlings may increase, % G and SVI-II are decreased but SVI-I increased suggest the healthy plantlet production with high biomass.

- ***In vitro* seed germination:** Plant tissue culture or the aseptic culture of cells, tissues and organs is an important tool in both basic and applied studies. Generally a single shoot arises from culture of one shoot tip but can be induced to develop multiple shoots. The isolated shoots can be rooted and complete plant recovered in large number and in lesser time (Narayanswamy, 1994). Shoots cultured on cytokinins characteristically develop into immature clusters rather than single axis. These shoots are then rooted to give rise to complete plantlets. Thus it is a means of unseasonable production of a large number of true to type plants *in vitro* in a short span of time. This has been carried out in many plant species. Similarly leaf cultures have been used for callus induction and regeneration of complete plantlets have been achieved in a number of plant species (Shekhawat et al., 1998, 2002).The final count of *W. somnifera* was 28-30 days *in vivo* germination. This period was decreased through tissue culture. WS-20 variety of *W. somnifera* was inoculated as an explant and the germination period was 15-17 days. Red light treatment *in vitro* was very much effective and development of 10-15 multiple shoots from the single seed. It was developed on plain MS medium. Each shoot was subcultured on MS medium supplemented with 10 mg/l KIN. Callus was also obtained from roots on radicals of seedlings on the same medium. It was of compact, tuft and brown coloured**.** Following are the output of results **(Table 3)**;

1. Shortening of time germination time period from 30 days to 7-10 days (almost saving of 75% timings) with tissue culture techniques.
2. 80-85% of *in vitro* germination in hormonal medium without red light but seedlings is also developed into compact callus.

3. 95-99% of *in vitro* germination and multiple shoots are obtained which may lead into individual plantlet formation with red light in hormonal medium.

CONCLUSIONS:

Following are the outputs from the aforesaid minor part of research work;

1. Healthy plants with high biomass but less % G with the treatment of PGR + Red light in *in vivo* germination.
2. Healthy plants with high biomass as well as multiple shoots & roots (which may lead a whole plantlet formation) are obtained with the treatment of PGR + red light in *in vitro* seed germination.

REFERANCES:

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.

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.

Jeong, B. R., Fujiwara, K. and Kozai, T. (1995): Environmental control and photoautotrophic micropropagation, *Hort. Rev.,* 17: 125-172.

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

Mehanna, H. T., Martin, G. C. and Nishijima, C. (1985): Effect of temperature and endogenous hormone content on peach seed germination and subsequent seedling growth, *Scientia. Hort.,* 27: 63-73.

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: 72-10.

Murashige, T. and Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Plant Physiol.,* 15: 473- 497.

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

Narayanswamy, S. (1994): Plant micropropagation, In: *Plant cell and tissue culture,* (ed.) Narayanswmy. S., Tata Mc Graw-Hill Publishing Company Limited, New Delhi, pp: 194-235.

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 - Recant Advances in Research and Technology,* Jan. 27-28, Jodhpur.

Shekhawat, G. S., Mathur, S. and Batra, A. (2002): An *in vitro* protocol for rapid propagation of *Azadirachta indica* A. Juss., *J. of Plant Biol.,* 29(1): 109-112.

Shekhawat, N. S., Mughal, M. H., Joshi, B. M. and Srivastava, P. S. (1998): Indian contribution to plant tissue and organ culture, In: *Plant tissue culture and molecular biology: applications and prospects,* (ed.) Srivastava, P. S., Narosa Publishing House, New Delhi, pp: 751-811.

Table 1: *In vivo* seed germination data of *Withania somnifera* (Variety: WS 20) under Without red light condition

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
Control	25	15	2	60	1.2	1.4	2.6	29.40	0.58	1.91	34.80	156.0
GA_3 10^{-5} M	25	18	0	72	1.5	1.0	2.5	28.61	0.51	2.15	36.72	180.0
GA_3 10^{-6} M	25	17	0	68	1.7	1.2	2.9	23.52	0.50	1.06	34.00	197.2
GA_3 10^{-7} M	25	11	0	44	1.6	1.1	2.7	30.60	0.52	1.14	22.88	118.8
KIN 10^{-5} M	25	19	0	76	2.2	1.2	3.4	26.30	0.65	1.75	49.40	258.4
KIN 10^{-6} M	25	13	0	52	2.5	1.0	3.5	23.90	0.60	1.58	31.20	182.0
KIN 10^{-7} M	25	14	0	56	3.0	1.0	4.0	24.60	0.59	1.22	33.04	224.0

Table 2: *In vivo* seed germination of *Withania somnifera* (Variety: WS 20) under With red light condition

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
Control	25	14	0	56	2.4	2.2	4.6	17.10	0.50	1.12	28.00	257.6
GA_3 10^{-5} M	25	15	0	60	3.1	2.2	5.3	29.06	0.53	1.44	31.80	318.0
GA_3 10^{-6} M	25	6	4	24	1.5	2.0	3.5	36.30	0.50	1.01	12.00	84.0
GA_3 10^{-7} M	25	9	1	36	2.0	2.1	4.1	51.10	0.83	0.97	29.88	147.6
KIN 10^{-5} M	25	17	1	68	2.9	2.1	5.0	29.40	0.59	1.80	40.12	340.0
KIN 10^{-6} M	25	10	1	40	1.7	1.7	3.4	26.20	0.40	1.10	16.00	136.0
KIN 10^{-7} M	25	5	5	25	1.7	1.8	3.5	33.00	0.70	0.95	17.50	87.5

Table 3: *In vitro* seed germination data of *Withania somnifera* (Variety: WS 20) under With and/or Without red light condition

Media	No. of Seedlings	% G	No. of Roots (days)				No. of Shoots (days)				Observation
			7	14	21	28	7	14	21	28	
Without Red Light											
Plain MS media	25	20	+	+	+	+	+	+	+	+	80 % G, Faster germination of seeds
MS + 10 mg/l KIN	25	21	+	+	+	+	+	+	+	+	84, % G, Faster germination of seeds, Seedlings developed into compact callus
With Red Light											
Plain MS media	25	24	+	+	+	+	+	+	++	++	96 % G, 5-7 shoots observed, Faster germination of seeds
MS + 10 mg/l KIN	25	24	+	+	+	+	+	++	+++	+++	96 % G, 10-15 multiple shoots observed, Faster germination of seeds