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# SEED GERMINATION STANDARDIZATION TECHNIQUES IN *WITHANIA COAGULANS* UNDER *IN-VITRO* AND *IN-VIVO* CONDITIONS, A CRITICALLY ENDANGERED MEDICINAL PLANT OF INDIAN THAR DESERT

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## Abstract

*Withania coagulans* is an important critically endangered medicinal plant of Indian Thar desert. It is commonly known as Indian cheese maker. The present paper deals with standardization of seed germination techniques under *in-vitro* and *in-vivo* conditions after application of different concentrations of growth regulators, sowing depths, soil mixture ratios, etc. Plant growth regulators such as $GA_3$, IAA, and IBA enhance the germination, growth and survival of seedlings. Results revealed that among all growth regulators, $GA_3$ and IAA are most prominent ones, which gave best results for seed germination and seedling growth.$GA_3$ are responsible for weakening of the seed coat so that the radicle of the seedling can break through the seed coat, whereas IAA promotes lateral and adventitious root development and growth. Under *in-vitro* conditions, optimum germination percent and seedling growth were observed when seeds were pre-treated with 5 $mgL^{-1}$ concentrations of $GA_3$. Maximum germination percent, *i.e.* 93.33 was reported after 9 days of seed sowing during December. The maximum seed germination percentage during rainy season were recorded when seeds sown at 1.5 cm depth with 1:2:1 soil mixture ratios of sand:clay : FYM. In 60 days old seedlings, root length and moisture were found highest in 1:1:2, whereas shoot length and total dry biomass in 1:2:1 soil mixture ratios.

***Key words :*** Germination values, growth regulators, soil mixture ratios, sowing depth, vigour index.

## Introduction

*Withania coagulans* (Stocks) Dunal (Family: Solanaceae) is common throughout Pakistan. It is also found in North-West India and Afghanistan. It is a perennial shrub and distributed in the East of the Mediterranean region and extends to South Asia. The plant is known by different names in various local languages such as 'Akri' or 'Puni-ke-bij' in Hindi, Spicebajja in Afghan, Khamjira in Punjabi and Punir band or Punirja-fota in Sindhi (Vaibhav *et al*., 2013). Jain *et al*., (2009) reported it as a critically endangered medicinal plant from the Indian arid zone. In Punjab, the fruits are used as the source of coagulating enzyme for clotting the milk, which is called 'paneer', so it is also known as "Doda Paneer". They are used in dyspepsia, flatulent colic and other intestinal infections. In some parts of Pak-Indian subcontinent, the berries are used as a blood purifier. Twigs are chewed for cleaning of teeth and smoke of plant is inhaled for relief in toothache (Gupta, 2012). It is used to treat nervous exhaustion, disability, insomnia, wasting diseases, failure to thrive in children, impotence, etc. The fruits are used for liver complaints, asthma and biliousness, while flowers in diabetes (Mathur and Agrawal, 2011).

Seed germination is one of the most crucial progressions in the plant endurance and progress among the stages of the plant life cycles in the arid and semi-arid regions of the world (Hadas, 2004). Before germination, seed fascinates water, subsequent in the enlargement and elongation of seed embryo (Singh *et al*., 2015). Seed germination is meticulous by numerous mechanisms and is essential for growth and development of the embryo, resulting in the eventual creation of a new plant. Under unfavourable circumstances, seeds may become dormant to sustain their germination capacity.

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However, when the situations are favourable seeds can germinate. There are number of aspects for controlling seed germination and dormancy, including plant growth hormones, which are produced by both plant and soil bacteria. Interactions between plant hormones and plant genes affect seed germination (Miransari and Smith, 2009).

Seed priming is advantageous practice to enhancement seed germination and growth in stress condition. Nowadays, various seed priming techniques have been advanced including hydro priming (soaking in water), halo priming (soaking in inorganic salt solutions), osmopriming (soaking in solutions of different organic osmotic) and bio priming (hydration using biological compounds), etc. (Ashraf and Foolad, 2005). Germination regulating compounds such as gibberellic acid ($GA_3$), kinetin and ethylene are known to alleviate the germination (Khan and Ungar, 2001). This alleviation in germination may be due to the stimulation of ATPase production, which rapidly increases during the early phases of germination to facilitate proton extrusion and $K^+$ uptake (Stout, 1988). Pre-sowing seed treatments with growth substances such as gibberellic acid have been found to improve the seedling growth of many species (Singh *et al*., 1989). Regulation of seed germination is closely related to the proportion between inhibitors especially ABA and growth regulators gibberellins (Hartmann *et al*., 1997). Auxin is a plant hormone present in the seed radicle tip during and after seed germination. The growth and development of different plant parts, including the embryo, leaf and root is believed to be controlled by auxin transport (Popko *et al*., 2010).

The arid zone is pre-dominantly occupied by sandy soils with very low water holding capacity with poor soil fertility and plant nutrients. The main fundamental of sandy soils is the sandy fraction and this does not supply any nutrient to the growing plants (Hamarashid *et al*., 2010). Clay is a very fine-grained soil of colloidal size comprising primarily of hydrated silicate of aluminium (Murali *et al*., 2018). Clays are the source of many of the chemical and physical properties of soils that make them a useful medium for the growth of plants. Farmyard manure (FYM) is one of the major sources of organic manures, which significantly influenced the soil organic matter concentration (%) in soil (Ojha *et al*., 2014). Application of FYM acts as a valuable source of plant nutrients and organic matter when incorporated into the soil (Khan *et al*., 2017).

Thus in the present studies, an attempt has been made to evaluate the effect of different concentrations of pre-treatments such as $GA_3$, IAA and IBA along with various soil mixture ratios of sand:clay : FYM and sowing depths to standardization the germination techniques and seedling growth parameters under *in-vitro* and *in-vivo* conditions in *W. coagulans*, an important critically endangered medicinal plant of the Indian arid zone.

## Materials and Methods

### Seed collections

The mature fruits of *W. coagulans* were procured from registered Ayurvedic medicinal shop during March-April 2016 & 2017. The seeds were cleaned and stored in plastic container with parad tablets (a mercury compound) to protect them from insect.

### *In-vitro* seed germination behaviour

Seed germination studies were conducted in freshly purchased and one-year-old stored seeds under *in-vitro* conditions. For germination studies, seeds were surface sterilized with 0.1% $HgCl_2$ solution for 30 seconds to avoid any infection and then kept under running tap water for 3-4 h to remove the adhering chemical particles. Afterwards, seeds were pre-treated with different concentrations, *viz.* 2, 5, 10 and 25 $mgL^{-1}$ of growth regulators such as $GA_3$, IAA, IBA by soaking them for 24 h. Distilled water alone was used for control set. Seeds were then kept in sterilized petridish lined with single layer of filter paper (Whatman No. 1) moistened with distilled water. Each petriplate contained 10 seeds and placed in seed germinator at $28^0$C temperature. Filter paper was moistened daily with 0.2 ml distilled water. Seed germination, root & shoot lengths and R/S ratio were recorded after 15 days of setting the experiments. Six replicates were maintained for each concentration and the experiments were repeated twice to confirm results.

### Germination Values and Vigour Index

Germination values of seeds were calculated for each treatment as per Czabator (1962):

$$GV = PV \times MDG$$

Where, PV= Peak value of germination; and MDG = Mean daily germination

PV is calculated with following formula:

$$PV = \frac{\text{Final germination percentage}}{\text{No. of days that took to reach the peak germination}}$$

MDG is calculated as follows:

$$MDG = \frac{\text{Total germination percentage}}{\text{Total No. of days}}$$

The vigour index (VI) was derived from the formula given by Abdul-Baki and Anderson (1973), which is as follows:

VI= Percentage of germination × Seedling length (cm)

Where, seedling lengths are the sum of root and shoot lengths.

***In-vivo* seed germination behaviour**

Seeds were sown under nursery conditions from January to December (2017 & 2018) to find out most suitable month for optimum germination. The percentage of seedlings emergence was recorded time to time. The best results for seed germination and seedling growth were obtained in 5 $mgL^{-1}$ $GA_3$ during December. The pre-treated seeds with 5 $mgL^{-1}$ $GA_3$ were sown during rainy season under *in-vivo* (nursery) conditions in thermo-cups (size: height 10 cm; bottom diameter 5 cm; and top diameter 7.5 cm) with different soil mixture ratios of sand:clay: FYM, *i.e.* 1:1:1, 1:2:1, 1:1:2 and 1:3:2 and sowing depths, *i.e.* 0.5, 1.0, 1.5 and 2.0 cm. For better growth performance, watering was done daily to keep the soil moist. Germination was recorded daily up to two months of setting the experiments. The different parameters such as plant height, root & shoot lengths and total dry biomass were recorded after 60 days of setting the experiments.

Experimental results were subjected to analysis of variance (ANOVA) using RBD as suggested by Gomez & Gomez (1984) and mean values of two years are presented in Tables 1-4.

## Results and Discussion

***In-vitro* seed germination behaviour**

The effect of different concentrations of growth regulator pre-treatments on various parameters of seed germination and seedling growth are presented in Table 1. Results revealed that fresh seeds showed maximum germination, *i.e.* 20.0% with 5 and 10 $mgL^{-1}$ concentrations of IAA and $GA_3$, respectively, whereas in one-year-old seeds with 25 $mgL^{-1}$ of IAA, *i.e.* 40.0%. IAA and $GA_3$ both are responsible for increasing maximum germination parameters in fresh seeds, whereas in one-year-old seeds with 25 $mgL^{-1}$ of IAA. Maximum root length was observed in 10 $mgL^{-1}$ of IAA in one-year-old seeds, while shoot length with control in fresh as well as old ones. Maximum R/S ratio was obtained in 2 $mgL^{-1}$ of IAA in fresh seeds. The maximum Germination Values (GV) were obtained in one-year-old seeds with control, while Vigour Index (VI) in IAA for fresh and one-year-old seeds. ANOVA revealed that in IAA treatment all parameters were significant in fresh seeds. Gibberellin stimulates seed germination via amylase synthesis (Finch-Savage and Leubner-Metzger, 2006). Chauhan *et al.* (2009) observed that 10 ppm $GA_3$ showed highest germination percentage in Black gram and Horse gram. IAA may not be necessary for seed germination, but is necessary for the growth of young seedlings (Hentrich *et al.*, 2013). Richa and Sharma (2003) observed that germination percentage increased in low concentrations of IAA and IBA, while declined with higher concentrations in *Cephalostachyum pergracile*. Similar results were reported by Paul *et al.*, (2008) in *Rauvolfia serpentina*, which also confirm our results.

Seed vigour is an important physiological seed characteristic (Baalbaki *et al.*, 2009). It is also govern the potential for high performance during seed storage and after sowing. Germination value is an index of combining speed and completeness of seed germination (Czabator, 1962). The germination value is closely related to the survival of seedlings (Djavanshir and Pourbeik, 1976). Dhoran and Gudadhe (2012) observed that $GA_3$ (60 ppm) showed highest vigour index in *Asparagus sprengeri*. Sagar and Kasera (2019) reported maximum values of GV and VI in 5$mgL^{-1}$ concentrations of $GA_3$ in *Dipcadi erythraeum*, which in accordance with our results.

***In-vivo* seed germination behaviour**

Effect of durations (months) on seedling emergence in *W. coagulans* was studied under nursery conditions (*in-vivo*) and the data are presented in Table 2. Data revealed that maximum germination percentage, *i.e.* 93.33 was observed after 9 days of sowing when seeds were sown during December. No germination was observed during May and June. Values for germination (%) were significant at $p<0.05$ level. The results on different sowing methods such as soil mixture ratios and sowing depths during rainy season under nursery conditions are presented in Tables 3. It is evident from this Table that minimum time to start germination was 8 days in 1:1:1 soil ratios at 0.5 cm depth. Maximum germination percentage was observed in 1:2:1 soil mixture ratios and 1.5 cm sowing depth, *i.e.* 66.66 and 51.11%, respectively. Seed sowing is defined as the process of placing the seed in soil to germinate and grow into plant. The basic objective of sowing operation is to put the seed in rows at chosen depth and spacing, shelter the seeds with soil and provide proper compaction over the seed (Jagtap *et al.*, 2016). Greven *et al.*, (2004) reported that best timing of sowing is an important factor since both seed immaturity and weathering reduce seed quality. Kasera and Shukla (2003) reported maximum germination in *Leptadaenia reticulata* when seeds were sown during May-June. Saharan *et al.*, (2001) reported maximum (50%) germination in *Evolvulus alsinoides* when the seeds were sown during 1st week of June to 1st week of July. Sagar and Kasera (2016) observed the maximum (80%) germination after 2 days of seed sowing during July in

**Table 1:** Effect of different concentrations of growth regulator pre-treatments on various parameters of seed germination and seedling growth in *W. coagulans* under *in-vitro* conditions.

| Growth regulators | Conc. ($mgL^{-1}$) | Germination (%) | | Seedling growth (cm) Root | | Seedling growth (cm) Shoot | | R/S ratio | | Germination values | | Vigour Index | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fresh | Old | Fresh | Old | Fresh | Old | Fresh | Old | Fresh | Old | Fresh | Old |
| Control | 0 | 6.66 | 16.66 | 0.95 | 1.0 | 3.25 | 2.4 | 0.29 | 0.47 | 0.03 | 4.62 | 27 | 56 |
| IAA | 2 | 3.33 | 26.66 | 0.3 | 0.66 | 0.1 | 1.78 | 3.0 | 0.48 | 0.049 | 3.15 | 1.33 | 65 |
| 5 | 20.0 | 20.0 | 0.95 | 1.26 | 2.03 | 2.28 | 0.46 | 1.06 | 1.904 | 2.05 | 59 | 70 | |
| 10 | 3.33 | 16.66 | 0.2 | 1.46 | 0.2 | 2.06 | 1.0 | 0.68 | 0.049 | 1.23 | 1.33 | 58 | |
| 25 | - | 40.0 | - | 0.89 | - | 2.19 | - | 0.64 | 0.052 | 1.26 | 0.99 | 123 | |
| CD | | 1.22** | 11.87** | 0.22** | ns | 1.42** | ns | 1.40** | ns | 0.11** | 1.22** | 1.40** | ns |
| IBA | 2 | 6.66 | 16.66 | 1.4 | 1.0 | 3.0 | 1.76 | 0.46 | 0.58 | 0.24 | 1.42 | 29 | 45 |
| 5 | 13.33 | 20.0 | 0.8 | 0.75 | 3.2 | 1.45 | 0.25 | 0.72 | 0.78 | 2.05 | 53 | 44 | |
| 10 | 13.33 | 13.33 | 1.15 | 0.65 | 1.87 | 1.6 | 0.61 | 0.77 | 0.84 | 0.78 | 40 | 29 | |
| 25 | - | 3.33 | - | 0.5 | - | 1.3 | - | 0.38 | - | 0.05 | - | 5.9 | |
| CD | | 1.22** | 11.02** | ns | ns | 1.42** | ns | 0.12** | 0.29** | 0.02** | 1.42** | 1.16** | ns |
| $GA_3$ | 2 | 16.66 | 13.33 | 0.72 | 1.22 | 1.4 | 1.77 | 0.51 | 1.11 | 1.23 | 0.91 | 35 | 39 |
| 5 | 6.66 | 6.66 | 0.8 | 1.05 | 1.8 | 0.85 | 0.44 | 1.75 | 0.19 | 0.11 | 17 | 12 | |
| 10 | 20.0 | 30.0 | 0.78 | 1.35 | 1.18 | 2.25 | 1.18 | 0.65 | 1.77 | 3.30 | 39 | 108 | |
| 25 | 6.66 | 16.66 | 0.2 | 0.76 | 0.1 | 2.26 | 2.0 | 0.33 | 0.05 | 1.42 | 1.09 | 50 | |
| CD | | ns | 11.02** | 0.25** | ns | 1.35** | 1.10** | 1.18** | ns | 0.15** | 1.71** | 1.22** | ns |

- = No germination; ns = Non-significant; and ** = Significant at ($P < 0.01$) probability levels.

**Table 2:** Effect of durations (months) on seedling emergence in *W. coagulans* under *in-vivo* conditions.

| Months | Day to start germinate | Germination (%) |
|---|---|---|
| January | 15 | 76.66 |
| February | 26 | 13.33 |
| March | 20 | 6.66 |
| April | 8 | 3.33 |
| May | - | - |
| June | - | - |
| July | 16 | 50.00 |
| August | 12 | 16.6 |
| September | 9 | 26.6 |
| October | 6 | 76.66 |
| November | 8 | 86.66 |
| December | 9 | 93.33 |
| CD | | 12.83* |

- = No germination; and * = Significant at ($P < 0.05$) probability levels.

*Dipcadi erythraeum*. In the present investigations, maximum germination was observed after 9 days of seed sowing during December as winter is the favourable season for optimum growth for this plant. During rainy season, the maximum values for germination percent was recorded at 1.5 cm depth with 1:2:1 soil mixture ratios of sand:clay : FYM.

It is clear from Table 4 that in 60 days old seedlings,

**Table 3:** Effect of different seed sowing methods on germination parameters in *W. coagulans* under *in-vivo* conditions.

| Seed sowing methods | | Days to start germinate | Germination (%) |
|---|---|---|---|
| Soil mixture ratios (sand:clay:FYM) | 1:1:1 | 8 | 42.00 |
| | 1:2:1 | 11 | 66.00 |
| | 1:1:2 | 9 | 64.00 |
| | 1:3:2 | 9 | 48.00 |
| CD | | | ns |
| Sowing depths (cm) | 0.5 | 10 | 42.22 |
| | 1.0 | 10 | 46.66 |
| | 1.5 | 10 | 51.11 |
| | 2.0 | 13 | 48.88 |
| CD | | | ns |

ns = Non-significant.

the highest values of root length and moisture were observed in 1:1:2 soil mixture ratios of sand:clay:FYM, whereas shoot length and total dry biomass in 1:2:1 ratios. Root& shoot lengths and total dry biomass were maximum when seeds were sown at 2.0 cm depth. The data of soil mixture ratios were significant, whereas non-significant for depths. The soil mixture ingredients and their proportion plays significant role in growth and development of seedling at nursery stage. Raising good quality seedlings at nursery stage is a very important aspect for success of any plantation programme (Vendrame *et al*., 2005). Kannur and Devar (2003) reported that soil having 1:1:2

**Table 4:** Effect of different sowing methods on various growth parameters in 60 days old seedling of *W. coagulans* under *in-vivo* conditions.

| Seed sowing methods | | Root length (cm) | Shoot length (cm) | Total dry biomass (mg $plant^{-1}$) |
|---|---|---|---|---|
| Soil mixture ratios (sand:clay:FYM) | 1:1:1 | 1.333 | 4.233 | 23.7 |
| | 1:2:1 | 8.166 | 5.333 | 55.1 |
| | 1:1:2 | 2.0 | 4.66 | 51.3 |
| | 1:3:2 | 1.566 | 3.166 | 25.2 |
| CD | | 12.89* | 7.890* | 8.127* |
| Sowing depths (cm) | 0.5 | 2.233 | 4.9 | 34.3 |
| | 1.0 | 2.666 | 4.566 | 32.7 |
| | 1.5 | 2.5 | 3.833 | 35.7 |
| | 2.0 | 3.5 | 5.333 | 47.8 |
| CD | | ns | ns | ns |

ns = Non-significant; and * = Significant at ($P < 0.05$) probability levels.

ratio of sand: soil: FYM is best for production of quality planting stock of *Spindus trifoliatus* in terms of superior growth and biomass. Swami and Kasera (2006) reported that seeds of *Withania somnifera* sown at 0.5 cm depth with 1:2:1 soil mixture ratios of sand:clay:FYM showed optimum germination, plant growth and biomass production. Saharan *et al*., (2001) observed maximum seedling emergence (70%) in soil having 1:2:1 ratio of sand:clay:FYM in *Evolvulus alsinoides*. Kasera and Shukla (2003) reported maximum germination percentage at 1.0 cm depth in *Leptadaenia reticulata*. Cent percent seedling emergence in *Asparagus racemosus* with 1:2:1 ratios of sand:clay:FYM was reported by Raghav and Kasera (2012). Sagar and Kasera (2016) observed that seeds of *D. erythraeum* sown at 1.5 cm depth with 1:1:2 soil mixture ratio of sand:clay:FYM showed optimum germination and seedling growth. In the present investigations, soil mixture ratios with higher clay and FYM contents and 1.5 cm depth were found to be most favourable for germination and seedling growth in *W. coagulans*, which was also supported by findings of above researchers.

## Conclusions

It can be concluded from the present studies that plant growth regulators have beneficial effects to stimulate germination in seeds of *W. coagulans*. $GA_3$ and IAA are most prominent ones, which gave best results for seed germination and seedling growth. Under *in-vitro* conditions, optimum germination and seedling growth were obtained when seeds were pre-treated with 5 $mgL^{-1}$ concentrations of $GA_3$. The seed sowing depth and different soil mixture ratios play a significant role in seed germination and seedling emergence. Winter is the most favourable season for maximum germination percent in *W. coagulans*. So, it is clear from the present research work that December is best month to grow *W. coagulans* plantlets under *in-vivo* conditions. Maximum germination percent was obtained at 1.5 cm depth with 1:2:1 soil mixture ratios of sand:clay : FYM during rainy season.

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