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Biology, Seed Morphology, Germination and Phytochemical Analysis of *Drimia indica* (Roxb.) Jessop from the Indian Thar Desert

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ABSTRACT

Drimia indica (syn. *Urginea indica*) commonly known as Jungli Piaz, is a medicinally important plant mainly used in chronic bronchitis and asthma. This article describes its seed/bulb morphology, germination behaviour and phytochemical composition. The seeds are heart-shaped and black coloured. Fresh as well as one-year-old seeds were hundred percent viable. Experimental study on the effect of growth regulators (GA_3 and IBA) on seed germination and seedling growth under *in-vitro* conditions, showed that maximum seed germination and root and shoot lengths and were obtained in seeds pre-treated with 5 mg L^{-1} concentration of GA_3. Phytochemical analysis revealed highest values of leaf pigments and total sugars in September, while maximum amount of crude protein, proline and phosphorus were recorded during July.

Key Words: *Drimia indica*; Seed Viability; Indian Arid Zone; Growth Regulators; Proline; Chlorophyll

INTRODUCTION

Plants have been an integral part of traditional medicine across the continent since time immemorial. Medicinal plants have their values in the substances present in various plant tissues with specific physiological action in human body. India is endowed with a rich wealth of medicinal plants. Plants are like natural laboratories where a great number of chemicals are biosynthesized and they may be considered the most important source of chemical compounds (Kirtikar and Basu 1995).

Drimia. indica (Roxb.) Jessop (Indian squill) is considered to have medicinal value and is largely used as an expectorant, cardiac stimulant, in treating rheumatism, dropsy, edema, gout, asthma, male sterility, psoriasis, chronic cough, swellings, pulmonary troubles, cardiac stimulant, in cutaneous, subcutaneous parasitic infections and as an anticancer agent. Due to these properties, the squill bulbs have found place in British and European Pharma-copoeias (Shiva Kameshwari 2013, Shiva Kameshwari et al. 2010). Despite these properties, the genus has not attracted much attention of the researchers in India. Due to unawareness and anthropogenic pressure causing habitat degradation much of genetic diversity is lost.

We have made an attempt to evaluate the morphological parameters of seeds/bulbs and effect of different concentrations of growth regulators on seed germination and seedling growth of *Drimia indica*. The application of gibberellin increases the seed germination as they increase the amino acid content in embryo and cause release of hydrolytic enzyme required for digestion of endospermic starch when seeds renew growth at germination. The overall development of plant is regulated by the growth regulators, nutrient and environmental factors. They also vary in their germination requirement (Chauhan et al. 2009). It is necessary to investigate the concentration of growth regulators suitable for seed germination and seedling growth.

Primary metabolites are of prime importance and essentially required for growth of plants. Many of these are precursors of pharmacologically active metabolites in pharmaceutical compounds such as antipsychotic drugs (Jayaraman 1981). In the present study, we analyzed primary metabolites such as leaf pigments, proline, total sugars, crude protein and phosphorus in this medicinal plant during different months.

MATERIAL AND METHODS

The plants were periodically surveyed in the field at Bhimbhadak, Jodhpur (15 km away north-west of the University Campus). The identity of plants was confirmed from the Botanical Survey of India, Jodhpur and specimens have been deposited in BSI herbarium (BSJO) bearing accession no. 35144.

During study period (2013-14), the average maximum and minimum temperatures were 34.86 and 19.25^{0}C, respectively. Average rainfall was observed 2.58 mm during February-March 2014. The soil of the study site (Bhimbhadak, Jodhpur) was basic in nature, sandy-gravelly in texture.

Mature seeds were collected during March 2014, cleaned and stored in plastic containers with parad tablets (a mercury compound) to protect seeds from insects until used under laboratory conditions. Seed size was measured by vernier calliper. Triplicate samples of 10 seeds each were used. Viability was assessed by tetrazolium method (Porter et al. 1947). The weight of 100 seeds was taken with the help of electronic balance.

Seed Germination Behaviour

For germination studies, seeds were surface sterilised with 0.1% $HgCl_2$ for 30 seconds to avoid any infection and then kept under running tap water for 3-4 h to remove the adhering chemical particles. Then, seeds were pre-treated with different concentrations, viz. 2, 5, 10 and 25 mgL^{-1} of GA_3 and IBA by soaking them for 24 h. Distilled water alone was used for control set. Seeds were then kept in sterilised Petri dishes lined with single layer of filter paper (Whatman No.1) moistened with distilled water. Each Petri plate contained 10 seeds and placed in seed germinator at 28^{0}C temperature. Filter paper was moistened everyday with 0.2 mL distilled water. Seed germination, root and shoot lengths were recorded after 7 days of setting the experiments. Six replicates were maintained for each concentration and the experiment was repeated twice to confirm results.

Phytochemical Analyses

For chemical analysis, leaf samples were collected randomly from the field during July-September 2013 and 2014. During rainy season average maximum and minimum temperatures recorded were 35.53 and 26.28^{0}C, respectively. Average rainfall was 103.13 mm. Plants were in leafless stage after rainy season, *i.e.* September onwards. Leaves were washed with running tap water to remove the adherent foreign particles, air-dried and used for chemical analyses. Proline, osmotic potential and leaf pigments were estimated in fresh leaf samples, while other parameters from oven-dried leaves. Fresh leaves were extracted with 80% acetone for estimation of leaf pigments as per Arnon (1949). Free proline was estimated as per Bates et al. (1973). Osmotic potential was estimated as suggested by Janardhan et al. (1975). Total sugars were estimated as per standard methods given by Plummer (1971) and Sadasivam and Manickam (1992). Nitrogen content was estimated by micro-Kjeldahl apparatus as suggested by Peach and Tracey (1955) and phosphorus as per Allen et al. (1976).

Statistics

The data collected during 2013 and 2014 were subjected to analysis of variance (ANOVA) using RBD as suggested by Gomez and Gomez (1984) with the help of SPSS version 16.

RESULTS AND DISCUSSION

D. indica is a perennial scapigerous herb. Bulb is tunicated, ovoid and thick with a diameter ranging from 29.4 to 72.0 mm (Figure 1A-B). The plant remains in vegetative state during rainy season (July-September), while flowering and fruiting during February to March, after which the leaves are shed (Figure 1C-D). Leaves are flat, linear and acute. Flowers are brown in colour and borne on scape. There are two types of plants: first, those with the bulb producing inflorescence without vegetative leaves and second, producing vegetative leaves along with inflorescence (Shiva Kameshwari et al. 2012). During field survey we have observed only the former one in which plant bears leaves in vegetative stage during rainy season and produces inflorescence during February to March after shedding the leaves. Perianths are in two whorls of three tepals each. Bracts are caducous, pedicels 2-3.5 cm long, spreading or recurved. Ovary is superior, syncarpous, trilocular, each locule has 1-2 ovules. Stamens are versatile and filaments are flattened. Fruit is ellipsoid capsule, 1-5 or more capsules per scape and has 4-21 seeds per capsule. Seeds are black, heart-shaped and flat (Figure 1E).

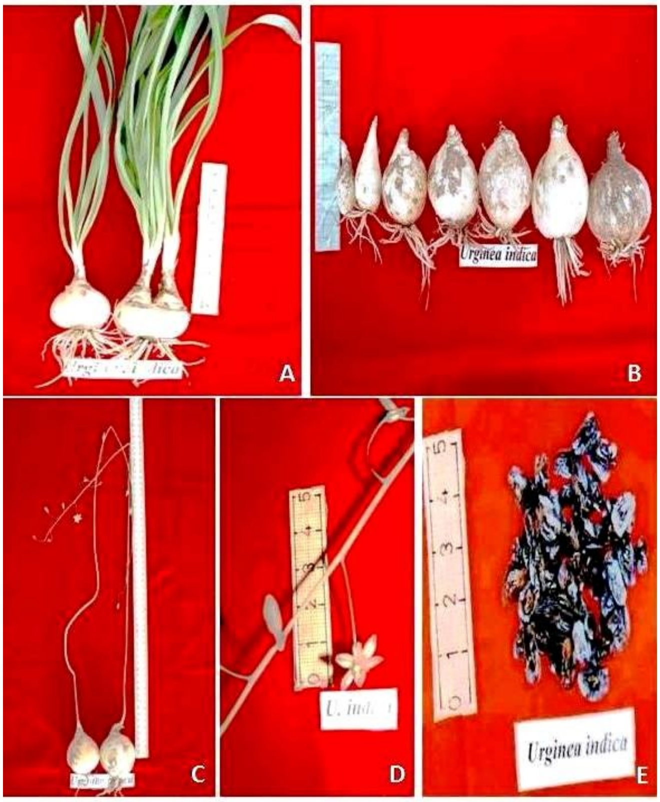

Figure 1. *Drimia indica* : A. uprooted bulbs with vegetative leaves from natural habitat, B. uprooted bulbs of various diameters, C. plant bearing flowers, D. close-up views of flower, and E. seeds.

The length and breadth of seeds varied from 7.5 to 11.0 and 4.6 to 6.5 mm, respectively (Table 1). Fresh as well as one-year-old seeds showed hundred percent viability. The diameter and weight of individual bulbs ranged from 29.4 to 72.0 mm and 58.5 to 208.0 g, respectively.

Data on seed germination (Table 2) reveal that seeds had 50.0% germination in control set. Hundred percent germination was observed with 5 and 10 mgL^{-1} concentrations of GA_3 and in 10 mgL^{-1} of IBA. Higher concentrations of both regulators retarded germination. Maximum root and shoot lengths were observed in 5 mg L^{-1} GA_3. Maximum R/S ratio was recorded when seeds were pre-treated with 10 mg L^{-1} of GA_3. Raghav and Kasera (2012) reported 100% germination in *Asparagus racemosus* Willd. seeds when pre-treated with 5 mg L^{-1} GA_3. The effect of IAA, IBA and GA_3 on *Melia azedarach* Linn. seeds was evaluated by Banerjee (1998) who observed that growth regulators were effective in increasing seed germination. Among these, GA_3 performed better than IAA and IBA, which also confirms our results. Statistical analysis of values revealed that data on all parameters were significant at $p<0.05$.

Data on phytochemical analysis of *D. indica* are presented in Table 3. Chlorophyll is the most indispensible primary compound as it captures sunlight for photosynthesis (Murray et al. 1986). We observed the highest value of total chlorophylls and carotenoids during September. Gehlot et al. (2012) observed maximum amount of total chlorophylls and carotenoids

Table 1. Morphological attributes of *D. indica* seeds and bulb.

Parameters		Values
Length (mm)		7.50-11.00
Breadth (mm)		4.60-6.50
Viability (%)	F	100.00
	O	100.00
Weight of 100 seeds (g)		0.38
Bulb diameter (mm)		29.40-72.00
Weght of bulb (g)		58.50-208.00

F= Fresh, and O= One-year-old seeds.

Table 2. Effect of different concentrations of growth regulator pre-treatments on various parameters of seed germination and seedling growth in *D. indica*.

Concentration (mg L^{-1})		Germination %	Root length cm	Shoot length cm	R/S ratio
Control		50.00	0.50	4.12	0.32
GA_3	2	60.00	0.53	5.96	0.09
	5	100.00	0.86	6.50	0.12
	10	100.00	1.16	4.98	0.23
	25	80.00	0.55	5.90	0.09
CD		15.75*	0.09*	0.10*	0.02*
IBA	2	80.00	0.42	4.12	0.10
	5	80.00	0.30	3.45	0.09
	10	100.00	0.20	2.08	0.10
	25	80.00	0.32	4.87	0.06
CD		15.75*	0.08*	0.22*	0.02*

* = Significant at ($P < 0.05$) level.

Table 3. Phytochemical parameters of *D. indica* in different months. Values are an average of six replicates.

Parameters	Months			CD
	July	August	September	
Chlorophyll *a* (mg g^{-1} f.wt.)	0.395	0.375	0.405	ns
Chlorophyll *b* (mg g^{-1} f.wt.)	0.261	0.252	0.268	ns
Total chlorophylls (mg g^{-1} f.wt.)	0.641	0.627	0.674	ns
Carotenoids (µg g^{-1} f.wt.)	0.350	0.305	0.394	ns
Proline (µg g^{-1} f. wt.)	1.118	0.634	0.968	ns
Osmotic potential (-MPa)	0.111	0.110	0.112	ns
Soluble sugar (mg g^{-1} d. wt.)	12.66	17.64	15.08	ns
Insoluble sugar (mg g^{-1} d. wt.)	9.5	8.29	12.58	ns
Total sugars (mg g^{-1} d. wt.)	22.16	25.93	27.66	ns
Crude protein (mg g^{-1} d. wt.)	5.394	4.603	3.022	0.84*
Phosphorus (%)	0.616	0.404	0.275	0.05*

* ns = Non-significant, and * = Significant at ($P < 0.05$) level.

during rainy season in *Withania coagulans* (Stocks.) Dunal. Kedia et al. (2008) documented maximum carotenoids in *Phyllanthus fraternus* Webster during rainy season. Our results confirm previous findings.

Maximum amount of proline accumulated during July. Osmotic potential slightly decreased when proline content increased and vice-versa. Proline accumulation is important for osmotic adjustment under abiotic stress (Hyun et al. 2003). Kasera and Shukla (2001) reported highest OP during rainy season and minimum during winter in *Leptadaenia reticulata* (Retz.) Wight & Arn. Sen et al. (2002) reported that proline accumulation in desert plants was accompanied by a decrease in osmotic potential, which is in accordance with present findings.

Total sugars values ranged from 22.16 to 27.66 mg g^{-1} dry wt. during three months, with a maximum in September when plants are in maturity stage. According to Mishra and Bhatt (2004) the sugar content in leaves increased with age and growth of plants. However, Kedia et al. (2009) observed maximum values of total sugars in rainy season during th vegetative stage in *Peganum harmala* Linn. that is in accordance with present study.

Proteins are the primary components of living beings. The presence of higher protein level in the plant indicates their potential higher food value or that a protein base bioactive compound could also be isolated in future (Thomsen et al. 1991). Crude protein and phosphorus were maximum in July and their values ranged from 3.022 to 5.394 mg g^{-1} d. wt. and 0.275 to 0.616 %, respectively. Mohammad et al. (2000) also recorded maximum crude protein in the leaves of *Trianthema triquetra* Rottler ex Willd. during rainy season.

Sagar and Kasera (2016) reported that the amounts of phosphorus were more at the time of new foliage formation followed by a gradual decrease with advancement of growing season in *Dipcadi erythraeum* Webb. & Berth. Similar observations reported by Naidu and Swami (1994) in *Terminalia arjuna* (Roxb.) Wight & Arn. confirm our results.

ANOVA revealed that data on crude protein and phosphorus were significant at $p<0.05$ and remaining parameters were not significant.

CONCLUSIONS

We conclude that growth regulators gave better responses over control. GA_3 performed best for seed germination and seedling growth but its higher concentration retarded them. IBA also showed inhibitory effect at higher concentrations. Phytochemical analyses revealed that leaf pigments and total sugars were accumulated in highest amount during September and other compounds in July. The study will help find most suitable developmental stage of plant for harvesting them to obtain these chemicals.

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