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Screening of traditionally used *Dactylicapnos scandens* (D.Don) Hutch. for the existence of secondary metabolites and development of propagation protocol

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ABSTRACT

Dactylicapnos scandens (D. Don) Hutch., Syn. *Dicentra scandens* (D. Don) Walp., is a perennial scandent herb that produces tuberous roots, the extract of which is used in traditional medicine to treat high blood pressure, asthma and to stop bleeding. The plant also has other medicinal uses. Although qualitative phytochemical analyses confirmed the presence of alkaloids, glycosides, terpenoids, and phenols, no flavonoids, saponins, and tannins were detected in tuber extracts. Seeds soaked in 100 ppm naphthalene acetic acid for 24 h recorded the highest rate of germination (50%), and those soaked in 200 ppm or 500 ppm gibberellic acid (GA_3) were the earliest to germinate (22.5 days). The frequency of sprouting was maximum (90%) in intact tubers (planted whole) whereas 6 cm long pieces of the tubers were the first to sprout (15.2 days). All the plants produced from tubers (whether from whole tubers or their pieces) recorded 100% survival at three months following transplanting.

1. Introduction

The genus *Dactylicapnos* of Fumariaceae has about 20 species of biennial and perennial climbing herbs distributed throughout the temperate region of North America and Eastern Asia (Stern, 1961, 1962, 1967). Hutchinson (1921) was the first person to monograph the genus, and later it was followed by Fedde (1936) in his taxonomic treatment of *Dactylicapnos* in his monograph of Papaveraceae. *Dactylicapnos scandens* (D.Don) Hutch. (Syn. *Dicentra scandens*) is a perennial climbing herb belong to family Fumariaceae and is commonly known as Yellow bleeding heart or locally Rhoodo by Chakesang tribes of Nagaland, India. The plant is known as Wandouxi in Wuliang Mountains of Jingdong, Yunnan, China where the whole plant is used in antiphlogosis, gastroenteritis, hypertension, haemostasis, traumatic injury (Gao *et al.* 2019). The plant grows well under temperate and sub-tropical conditions. It is naturally distributed in the temperate and subtropical region of the Central and Eastern Himalayan region viz. Bhutan and Sikkim (Grierson and Long, 1984), Nepal and Nagaland between altitudes range from 1500-2500m amsl and prefers cool climatic conditions; hot and dry weather is unfavourable for growth and development of the plant. The dried tuber of the species is a well-known traditional Chinese medicine (TCM) with the Chinese name "Zijinlong", and is used for the treatments of hypertension, inflammation, bleeding and pain for centuries (Guo *et al.* 2013). *D. scandens* has been used traditionally as an important ethnomedicinal plant by certain tribal communities of North East India and Nepal (Pfoze *et al.* 2010). The plant is locally called as Jensüng Khaonying and its leaves are taken as raw to cure asthma and the paste of the rhizome is applied during insects and snake-bite (Jamir *et al.* 2012). The extract of the plant is prescribed as a remedy against fevers, high blood pressure, gastrointestinal disorders and diuretic. The paste is applied on cut and injury to control bleeding and for wound healing. The paste made from the crushed root tuber is also applied to get relief from toothache (Pfoze and Chiezou, 2006). Some tribes of Arunachal Pradesh use the paste from the fresh tuber to treat snake bite and the powder prepared from dried tubers is

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used in gastric problem (Hajra *et al.* 1996). In Nepal, the plant is fed to animals as an anthelmintic medicine and is also used in religious ceremonies such as Shradda i.e., the rituals and offerings to the dead (Manandhar and Manandhar, 2002). The plant is known as Jogilahara locally by Limboo tribes of Sikkim and the dried root powdered is used orally during gastritis (Badola and Pradhan, 2013). The boiled decoction of the crushed root is given to stop excessive bleeding in females by Lepcha tribes of Sikkim (Pradhan and Badola, 2008). Although the species is very important from its medicinal use point of view and popular among some tribes of North-eastern state of India, very little work has been done on the species regarding its qualitative analysis and propagation techniques. Therefore, the present study was proposed to generate more information on the propagation to help out the farmer community for the large-scale production and preliminary screening (qualitative) was done for the identification of the essential secondary metabolites to find out the active chemical compounds present in *D. scandens* tubers extracts.

2. Methodology

Collection of the planting material

Tubers of *D. scandens* were procured from ICAR Research Complex for North Eastern Hill Region, Umiam, Meghalaya, and plants were successfully raised in the field of the Department of Forest Products and Utilization, College of Horticulture and Forestry, Central Agriculture University, Pasighat (28°04'43" N, 95°19' 6" E; elevation, 153 m).

Preliminary screening of secondary metabolites

To identify the chemical constituents of the tubers, namely alkaloids, saponins, terpenoids, flavonoids, glycosides, tannins, phenols, and steroids, a suspension of 0.5 g of the dried tuber was extracted in 10 mL of distilled water and was filtered using Whatman No.1 filter paper. The filtrate was used for the determination of the constituents using the tests mentioned in Table 1.

Seed germination studies

Matures seeds collected from field grown plants in October /November was tested for germination parameters under laboratory conditions. Seeds was treated with various concentrations of GA_3, IAA and NAA and were sown in petri-dishes containing filter paper at 20 ± 1°C in seed germinator and observations were recorded on germination percentage, peak germination period, germination energy and germination value.

Vegetative propagation studies

Tubers of *D. scandens* were harvested from the field, washed thoroughly under running tap water to remove soil particles, and cut into pieces of uniform thickness but of different lengths- 2 cm, 4 cm, 6 cm, 8 cm, and 10 cm. The whole tubers served as a control. These were planted in January 2019 in a greenhouse. The number of tubers that sprouted was recorded to calculate the sprouting percentage. Survival percentage was calculated by recording the number of plants that survived up to three months from planting.

Statistical analysis

The experiment for the seed germination was laid under Completely Randomized Design (CRD-Factorial) and for tubers sprouting, it was under Randomized Block Design (RBD) and data were analyzed using one way analysis of variance (ANOVA).

3. Results

Preliminary screening of secondary metabolites (Qualitative) of *Dactylicapnos scandens* tubers extract

The preliminary screening of secondary metabolites showed the presence of alkaloids, glycosides, terpenoids, and phenols but no tannins, saponins, flavonoids, or steroids were detected (Table 1).

Table 1. Preliminary screening of secondary metabolites (qualitative) in the tubers of *Dactylicapnos scandens*

SN	Secondary metabolites	Test	Results
1	Alkaloids	Wagner's test	+
2	Tannins	Ferric chloride test	-
3	Phenol	Ferric chloride test	+
4	Glycosides	Ellagic acid test	+
5	Flavonoids	Alkaline Reagent Test	-
6	Terpenoids	Salkowski's Test	+
7	Steroids	Salkowski's Test	-
8	Saponins	Foam test	-

In the above experiment, we have observed that the species contains secondary metabolites that are of great value for the medicinal purposes. They are related to the traditional practices for treating diseases from the plant extract. However, the plant is being used by the tribal communities in the large scale but they don't have sufficient knowledge regarding the propagation protocol. Therefore, they always face difficulty in multiplying the plant. As per perusal of literature it is revealed that under natural conditions the species has very low seed germination. Therefore, to solve this problem we have experimented to know about the propagation of the plant by both seed and vegetative (tubers) method.

Influence of plant growth regulators on seed germination in *Dactylicapnos scandens*

Germination percentage

The growth regulators affected germination significantly (Figure 1). Germination percentage ranged from 0% to 33% and from 0% to 50% when the seeds had been soaked for 12 h or 24 h, respectively. Maximum germination in both cases being in seeds treated with 100 ppm NAA. However, the differences due to the duration of soaking or those due to the interaction between the growth regulator and the duration were not statistically significant.

Peak germination period

Neither the treatments nor the interaction between the growth regulator and the duration had any significant effect on peak germination period.

Germination energy

The growth regulators showed a significant effect on germination energy (Figure 1), whereas the duration of soaking had no significant impact. Maximum germination energy (25%) was recorded in seeds treated with 100 ppm NAA.

Germination value

Neither the treatments nor the interaction between the growth regulator and the duration had any significant effect on germination value.

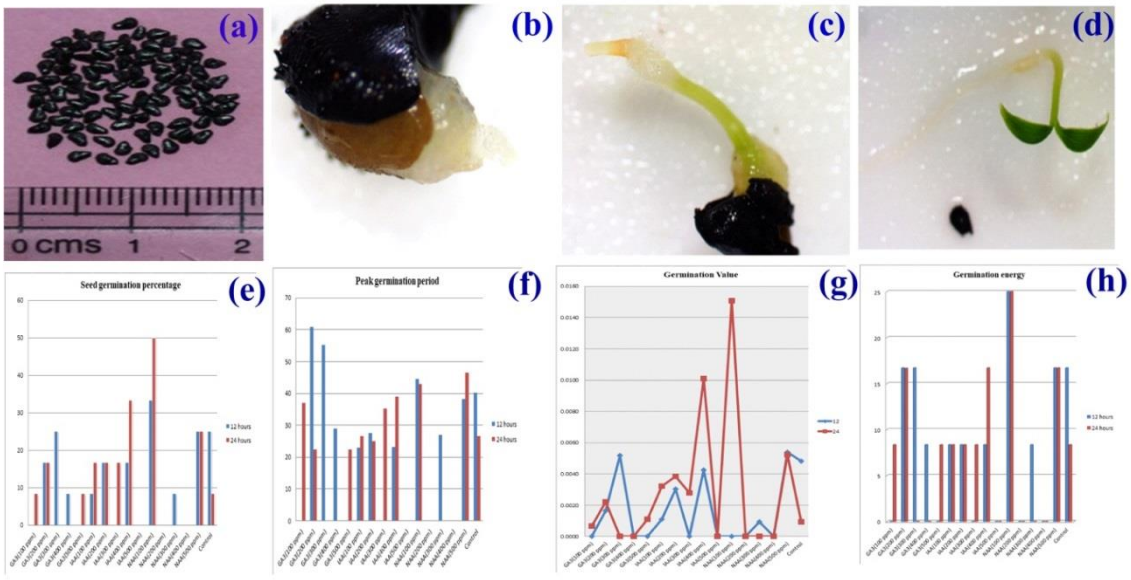

Fig. 1 - (a) Seeds; (b) Splitting of seed coat; (c) Development of Radicle, epicotyl, hypocotyl; (d) Development of plumule; (e Seed germination percentage influenced by growth regulators and time periods; (f) Peak germination period influenced by growth regulators and time periods; (g) Germination energy influenced by growth regulators and time periods; (h) Germination value influenced by growth regulators and time periods

Influence of division of tubers on multiplication and development of *Dactylicapnos scanden*

Tables 2. Corresponding sequence of events in the development of *Dactylicapnos scandens*

Events	Date	Time Taken
Planting of tubers	10/01/2019	–
Sprouting	23/01/2019	14 days
Transplanting	01/02/2019	22days
1st inflorescence	19/05/2019	130 days
1st flowering	22/05/2019	134 days
Flowering period	June-October	143 - 294 days
1st Fruiting	10/06/2019	154 days
Ripening of fruiting	June –October	143 - 294 days

Table 3. Effect of length of tubers on sprouting and survival

Treatment	Number of tubers sprouted	Sprouting percentage (Angular transformation)	Days taken for sprouting	Survival percentage
Treatment 1 (undivided)	18 (12.20)	90% (76.70)	29.25	100%
Treatment 2 (2 cm cutting)	8 (7.99)	40% (38.93)	30.25	100%
Treatment 3 (4 cm cutting)	12 (9.90)	60% (51.03)	19.00	100%
Treatment 4 (6 cm cutting)	11(9.50)	55% (47.86)	15.25	100%
Treatment 5 (8 cm cutting)	14 (10.80)	70% (57.07)	26.25	100%
Treatment 6(10 cm cutting)	12 (9.90)	60% (51.03)	29.75	100%
C.D. (5%)	1.818	14.52	3.79	NS

Pieces of tuber 6 cm long were the first to sprout (15 days), followed by those 8 cm long, whereas the smallest tubers (2 cm) were the last to sprout.

Sprouting percentage

The length of the tuber pieces had a significant effect on the proportion of tubers that could sprout. The proportion was the highest (90%) in intact tubers (Table 3), followed by pieces of tubers 8 cm long. For mass multiplication, the tubers should be cut into pieces 8 cm long.

Survival percentage

All the tubers, whether intact or cut into pieces of varying length, produced viable plants (Figure 2). The survival percentage after three months planting was 100%.

4. Discussion

The results of the analyses of the extract of tubers for secondary metabolites matched those reported by (Nakhuru *et al.* 2013). The marked effect of growth regulators on seed germination observed in the present study is consistent with that seen in different species of *Dicentra* as reported by Atwater (1980) in *D. chrysantha*) and Keeley and Fotheringham (1998) in *D. ochroleuca*, who observed that 400 ppm GA_3 stimulated germination. However, in *D. chrysantha*, scarification alone induced germination; GA_3 did not affect either alone or when combined with scarification because small or rudimentary embryos are the characteristics of the species. In the present investigation, NAA proved the most effective in promoting the germination of seeds of *D. scandens*. Although undivided tubers have more buds than divided tubers and resulted in a higher sprouting percentage, using 8 cm long pieces of tubers, that recorded 70% sprouting and 100% survival of the plants, will be more economical for large-scale multiplication. The favourable effect of dividing the tubers on sprouting and survival was also reported in *D. spectabilis* (Kaminska *et al.* 2005).

5. Conclusion

Based on the results of using seeds and pieces of tubers as propagules, it is recommended that pieces of tubers 8 cm long be used for propagating *Dactylicapnos scandens*, which needs to be multiplied on a large scale given its medicinal value.

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Fig. 2 - (a) Tubers; (b) Tubers Divisions; (c) Sprouting of tuber; (d) *Dicentra scandens* flowers and fruits

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