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In vitro micrpropagation of *Tacca leontopetaloides* (L.) kuntze

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Abstract
Tacca leontopetaloides (L.) Kuntze belongs to family Taccaceae. It is highly medicinal plant used for stomach disorders, diarrhea, and dysentery also used in worm infection, hepatitis, and snake bites. It is rich source of carbohydrates, alkaloids, vitamin-C, vitamin-E, flavonoids, and other primary and secondary metabolites such as Phenols, glycosides, saponins, and volatile oils are present. The aim of this study was to micropropagate the *Tacca leontopetaloides* by using leaf and shoots tip as an explant. The explants were inoculated with the sterilized MS-media with different concentrations of Cytokinin and Auxin i.e. BAP and IAA. The combination of 2.0 mg/l of BAP with 1.0 mg/l of IAA (1.92 ± 0.216) and a combination of 2.5 mg/l of BAP with 1.0 mg/l of IAA (2.0 ±0.144) was proved to be a maximum number of shoots with leaf was used as an explant. Maximum shoot was observed on media with a combination of 2.0 mg/l of BAP with 1.0 mg/l of IAA (2.64 ± 0.203) and a combination of 2.5 mg/l of BAP with 1.0 mg/l of IAA (2.78± 0210) with shoot tip used as an explant as compared to when leaf used as an explant.

Keywords: *In-vitro* micropropogation, explant, *Tacca leontopetaloides* (L.) kuntze

Introduction
Tacca leontopetaloides (L.) Kuntze is a perennial wild plant of the Taccaceae family. Tacca J. R. & G. Forst is the only genus in the Taccaceae family, which is a relatively recent plant family formed out of the Dioscoreaceae, however the two families are still taxonomically interrelated (Borokini, 2014) [3]. The plant is native to Malaysia and the Pacific Islands, however it may now be found from western Africato northern Australia via Southeast Asia. The tubers were used as staple food in Polynesia, as well as a source of carbohydrates (Ogbonna, 2017) [13]. The tubers of *Tacca leontopetaloides* are an important food source for baking food materials like wheat flour, bread, pastries, pudding, and mixed with different ingredients like coconut cream, coconut juice, or fruit pulp (Lim, 2016). The tuber is used for stomach disorders, diarrhea, and dysentery, also used in worm infection, hepatitis, and snake bites. It can be fermented to make alcohol (Borokini, and Ayodele, 2012) [2]. The tuber part is mostly used to treat gastric ulcers, toothache, Enteritis, and sexual dysfunction, stomach, disorders (Conrad *et. al*., 2018). Tuber paste is used on boils and hairs on the nearby portion (Ubwa *et. al*., 2011) [17]. Tubers are high in carbohydrates, alkaloids, vitamin-C, vitamin-E, flavonoids, and other primary and secondary metabolites, according to phytochemical screening. Phenols, glycosides, saponins, and volatile oils are some of the compounds found in plants (Jagtap, 2014) [7]. Antioxidants contain flavonoids and saponins. They protect cells from the harm caused by free radicals. In most situations of chronic illnesses like cancer and diabetes, they can act as a mediator (Jagtap, 2015) [8]. Spirostanol saponins are present in *Tacca leontopetaloides* which are used for reduction of cholesterol (Jiang, 2014 and Aissatou, 2017) [9]. The *in vitro* micropropogation of *Tacca leontopetaloides* was undertaken become of its poor seed germination.

Materials and Methods
Preparation of media and growth condition
MS (Murashige and Skooge, 1962) medium was utilized as the basal medium for *in vitro* experiments. It has a balanced composition of macronutrients, micronutrients, vitamins and organic supplements that are particularly required for the majority of plant species. The experimental material of *Tacca leontopetaloides* was collected from the Ambabarwa forest, district-Buldhana. Fresh leaves were collected from the plants. Explants washed with running tap water and were surfaces sterilized with 0.3 % (w/v) $HgCl_2$ (RFCL Ltd, India). These explants were washed with sterile distilled water for 4-6 min; leaves were cut into pieces (1.5 to 2.0 cm.) and inoculated on MS- Media supplemented with 3% sucrose and a different combination of Auxins and Cytokinins i.e. IAA and BAP was added.

Process of inoculation
The inoculation is carried out under a clean bench equipped with a laminar air flow of ultraviolet (UV) tubes and fluorescent tubes**.** The surface of the chamber was cleaned with 90% alcohol.

Culture condition
This research work was carried out under controlled conditions. MS medium supplemented with 3 % sucrose and 0.3 Clerigar. After the addition of growth regulators pH was adjusted to 5.8 the medium was sterilized in an autoclave at 15 psi pressure and 121^0C temperature for 20 min. The sterilized medium was transferred into laminar air flow chamber for inoculation process. After inoculation, culture

vessels were placed in a growth room having 25 ± 2^0 C temperatures for 3-4 weeks with 16 hours of photoperiod and 70% relative humidity. Subculture was done every 3 to 4 weeks.

Data was recorded after every week and analyzed by five replicates for shoot multiplication and shoot length with mean ± SE.

Results and Discussion

MS medium in various concentrations with IAA values of 1.0, 1.5, 2.0, 2.5, and 3.0 mg/l and BAP was used to maximum average percentage of shoot multiplication. For shoot multiplication, combination of BAP and IAA produced better results (Table 1).

Table1: Effect of various concentrations of Cytokinin of BAP and IAA on induction of shoots multiplication from leaf and shoot tip of plant.

Concentration of plant growth regulators (PGRs) (mg/l)		Leaf		Shoot tip	
	IAA	No. of shoots / explant	Shoot Induction (%)	No. of shoots / explant	Shoot Induction (%)
BAP 2.0	1.0	1.92 ± 0.216	56	2.64 ± 0.203	60
	1.5	1.44 ± 0.151	42	1.76 ±0.258	46
	2.0	1.42 ± 0.044	38	1.58 ± 0.083	38
	2.5	1.28±0.083	34	1.52 ± 0.083	36
	3.0	1.16 ± 0.054	32	1.34 ± 0.054	34
BAP 2.5	1.0	2.0 ± 0.144	60	2.78 ± 0.210	78
	1.5	1.44 ± 0.050	58	1.92 ± 0.037	70
	2.0	1.34± 0.040	52	1.52 ± 0.048	64
	2.5	1.32± 0.037	40	1.32 ± 0.040	62
	3.0	1.28 ± 0.040	36	1.32 ± 0.020	52

Values represent mean ± standard error (SE) based on five replicates and percentage based on two experiments each of five replicates.

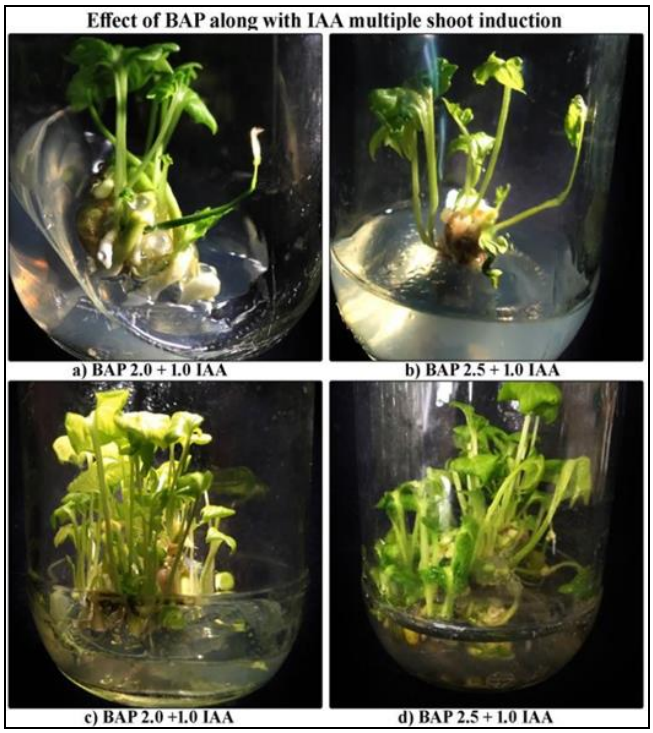

Photo Plate 1: Effect of BAP along with IAA for multiple shoot induction.

The aim of the study was to generate the *Tacca leontopetaloides* plant saplings through micro-propagation by using leaf and shoot tip as an explant. The effect of MS medium with various concentrations of BAP with IAA has been studied. The response of shoot regeneration with explants to various concentrations of BAP (2.0-2.5) and (1.0-3.0) shows in Table No.1

Higher-level of multiple shoots regeneration was achieved at BAP 2.5 mg/L with IAA 1.0 mg/L with the maximum number (2.0 ± 0.144) with 60% of shoot regeneration with leaf as an explant (photo plate-1.c). Decreased the number of shoots with the increased level of IAA 3.0 mg/L with leaf explant.

Shoot tips were inoculated on MS medium with 2.5 mg/L BAP and 1.0 mg/L IAA this combination was shown effective which showed maximum number of shoots (2.78±0.210) and frequency 78% shoots regeneration (photo plate-1.d).

Conclusion

The higher level of Auxin concentration was not found to be suitable for the maximum number of shoot multiplication. The 2.5 mg/L BAP with 1.0 mg/L IAA showed favorable composition for initiation of multiple shoot regeneration from leaf and shoot tip as an explant.

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