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REGENERATION POTENTIAL OF RIGHT AND LEFT COTYLEDONS IN WINGED BEAN (*PSOPHOCARPUS TETRAGONOLOBUS* (L.) DC.)

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ABSTRACT

Psophocarpus tetragonolobus (L.) DC. (Winged bean), the wonder crop of the new millennium, is highly rich in proteins and essential amino acids and can be a boon to the developing nations to overcome the problem of malnutrition. It is a hot crop for tissue culturists, as it is recalcitrant to tissue culture techniques. The present study was conducted to evaluate the regeneration potential of right and left cotyledons separately as explants for in vitro propagation of the plant. The proximal and distal halves of both right and left cotyledons were also studied individually for assessing their regeneration potential. The right cotyledon gave maximum response of 17 shoots in MS media containing IAA (0.2 mg/l) along with BAP (2 mg/l) while the left cotyledon gave only 7 shoots in the same media composition. When the proximal half of the right and left cotyledon were used as explants, a similar response was observed. But when the distal halves were used, only callusing was obtained in both the cases.

KEYWORDS: Clonal Propagation, Cotyledon Culture, *Psophocarpus tetragonolobus*, Winged Bean

INTRODUCTION

Global food security is increasingly based on a narrow range of animal and plant species. About 95% of the worlds' food energy needs is provided by just 30 plant species and 50% of the requirement for protein and calories is met just by maize, wheat and rice [3]. But there are many underutilized crops that can make important contributions to the nutrition and health of people in developing countries. The species of leguminosae includes very important vegetable crops due to its high protein content in seeds as well as in tubers and the moderate oil content in its seeds. *Psophocarpus tetragonolobus* (L.) DC., commonly known as 'winged bean' is an underutilized leguminous crop, which received lessresearch attention since emphasis had been on cash crops. An outstanding feature of winged bean is that it contains significant amount of protein in all parts of the plant i.e., seeds, pods, leaves and roots or tubers, all of which are edible. The tubers contain 20% protein in dry weight. This amount is superior to other tubers like yam (2%), cassava (1%), potato (2%) and sweet potato (2%). The percentage of crude protein of the seeds (29.8-37.4%) is comparable to soybean seeds (25%) and is higher than that of other legumes [10].

Legumes in general are recalcitrant to tissue culture and are highly genotype specific [13]. Since the members of the family Leguminoseae contain high levels of phenolics, plant recalcitrance caused by the oxidation of explants is highly prevalent [1]. By alleviating the negative effects of this stress, modified regeneration methods are being reported in legumes by various tissue culture routes. Eventhough, *Psophocarpus tetragonolobus* (L.) DC. is usually considered to be recalcitrant to tissue culture techniques, there are some reports on successful tissue culture protocols in winged bean [2,6,8,15]. Cotyledons, though have been found to be good explants for successful *de novo* organogenesis, the present study was carried out with an objective to evaluate the efficacy of the use of the right and left cotyledons separately as explants than using the whole seed as explant.

MATERIALS AND METHODS

Seeds were obtained from Kerala Agricultural University, Mannuthy, Thrissur, India. The seeds were dipped in a liquid detergent (Teepol) for five minutes and kept in running tap water for two hours. The seeds were then transferred to the laminar air flow chamber and surface sterilized with 0.1% mercuric chloride for seven minutes and then washed thoroughly in double distilled water for 5-7 times to remove all the traces of the sterilant. The seeds were scarified with 80% sulphuric acid for 3 minutes to remove the hard seed coat. Then the cotyledons were washed properly with sterile distilled water to remove all the traces of the acid. The cotyledons were separated and the cotyledon that was to the right when the intact cotyledon was placed facing front with the embryonal axis on the upper side was designated as the right and the other as left cotyledon. Care was taken to remove the embryonal axis completely before inoculation. The whole right and left cotyledons as well as the proximal and distal halves of the right and left cotyledons were also used as explants. The explants were inoculated onto MS media supplemented with 1-5 mg/l BAP/KIN alone or in combination with 0.2-2 mg/l of IAA/NAA/IBA to determine their efficacy in shoot morphogenesis.

RESULTS

The right cotyledon gave 6 shoots/ explant and the left cotyledon gave 2 shoots / explant when inoculated in BAP (2 mg/l) containing medium (Table 1). There was a decrease in the number of shoots produced per explant, when the concentration of BAP was increased above 2 mg/l. No response was observed when the concentration of BAP was increased to 5 mg/l. There was a similar pattern of response when the right and left cotyledons were inoculated in KIN containing media, but with reduced number of shoots (Table 1). The addition of an auxin (IAA/ NAA/ IBA 0.2 -2 mg/l) along with BAP (1-5 mg/l) gave a significant increase in the number of shoots initiated (Table 1). IAA (0.2 mg/l) + BAP (2 mg/l) containing MS media gave the maximum response with 17 shoots from the right cotyledon and 7 shoots from the left cotyledon (Figure 1). The number of shoots produced decreased when the concentration of either IAA or BAP was increased. The proximal halves of both the right and left cotyledons also showed a similar response with maximum response being in MS media containing IAA (0.2mg/l) + BAP (2 mg/l) from the right proximal half. The distal halves of both the right and left cotyledons resulted only in callusing in all the combinations tried. The other combinations of NAA (0.2-2 mg/l) + BAP (1-5 mg/l) and IBA (0.2-2 mg/l) + BAP (1-5 mg/l) were inferior (Table 1) when the right and left whole cotyledons as well as the proximal and distal halves of the right and left cotyledons were inoculated. Elongation of shoots along with rooting was obtained in the same medium.

DISCUSSIONS

Maximum shoot regeneration from cotyledon explants was observed in an auxin + cytokinin containing media. Previous reports on leguminous species have showed maximum regeneration potential of the cotyledon being exploited in a cytokinin alone containing medium [9,15], whereas the present study showed that the addition of an auxin (IAA/NAA/ IBA) in low concentrations (0.2 -0.4 mg/l) along with BAP (2 mg/l) was necessary fortapping the morphogenetic potential of the cotyledon successfully. The variation in response between the right and left cotyledons to various hormonal regimes might be due to the strong attachment of the right cotyledon to the embryo than the left cotyledon. The proximal half of both the right and left cotyledon only responded in shoot formation and the distal halves resulted in callusing. This variation in response of the proximal and distal halves of the cotyledon corresponds with the observation in sunflower [4] and ground nut [14]. Sharma *et al.*[12] suggests that a diffusible growth factor promoting somatic embryogenesis emanates from such explants. Such a growth factor has been shown to reside in the radicular halves of the cotyledons of *Cassythafiliformis*[11]. This diffusible factor has been suggested to be an auxin like substance that in the presence of a cytokinin, activates the totipotent cells for bud formation in *Glycine max*[5], *Cucumissativus*[7] and *Brassica juncea*[12]. The correct hormonal regime used might have induced meristematic nodule and subsequently shoot primordial formation from these meristematic areas. This explains the morphogenetic competence of the proximal end of the cotyledons of *P. tetragonolobus* as observed in the present study.

CONCLUSIONS

From the results obtained, it can be concluded that the use of the right cotyledons as explants for the rapid propagation of *Psophocarpus tetragonolobus* is highly preferable. Further, the proximal halves of the right and left cotyledons are also suitable for rapid propagation, while the distal halves cannot be used for the same.

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APPENDICES

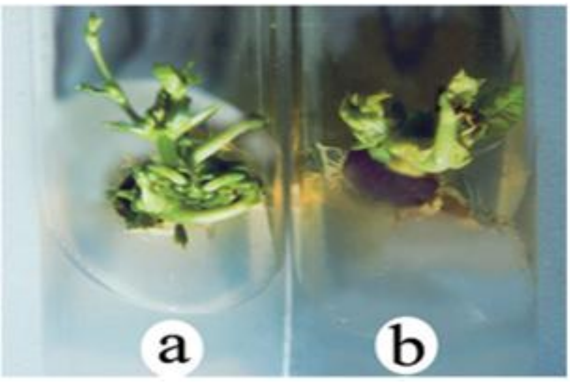

Figure 1: Shoot Proliferation from Right (a) and Left (b) Cotyledon after 30 Days

Table 1: Effect of Plant Growth Regulators on Shoot Formation from Right and Left Cotyledons

Hormones (mg/l)					Mean No. of Shoots ± S.E	
IAA	**IBA**	**NAA**	**BAP**	**KIN**	**Right Cotyledon**	**Left Cotyledon**
-	-	-	2	-	6.14 ± 0.26^{e}	2.00 ± 0.00^{ef}
			4		2.14 ± 0.40^{f}	1.86 ± 0.14^{ef}
-	-	-	-	2	2.86 ± 0.40^{f}	1.29 ± 0.18^{fgh}
				4	1.86 ± 0.40^{f}	-
0.2	-	-	2	-	17.29 ± 0.97^{a}	7.14 ± 0.40^{a}
0.4			2		12.14 ± 0.55^{c}	6.00 ± 058^{b}
0.2	-	-	4	-	15.86 ± 0.55^{b}	4.86 ± 0.51^{c}
0.4			4		10.86 ± 0.59^{c}	3.57 ± 0.37^{d}
-	0.2	-	2	-	8.29 ± 0.71^{d}	2.57 ± 0.20^{e}
	0.4		2		6.00 ± 0.31^{e}	1.43 ± 0.20^{fg}
-	0.2	-	4	-	5.71 ± 0.36^{e}	1.29 ± 0.18^{fgh}
	0.4		4		2.86 ± 0.26^{f}	0.57 ± 0.20^{gh}
-	-	0.2	2	-	5.43 ± 0.30^{e}	0.43 ± 0.20^{h}
		0.4	2		3.14 ± 0.26^{f}	+
-	-	0.2	4	-	4.71 ± 0.61^{e}	+
		0.4	4		2.43 ± 0.48^{f}	+
					Proximal Halves	
0.2	-	-	2	-	7.82 ± 0.40^{de}	2.71 ± 0.36^{e}
0.4			2		5.29 ± 0.97^{e}	1.29 ± 0.18^{fgh}
0.2	-	-	4	-	6.43 ± 0.48^{e}	1.14 ± 0.40^{fgh}
0.4			4		2.71 ± 0.36^{f}	0.43 ± 0.20^{h}

Note: Values are means of 7 replicates. Mean values followed by the same letters are not significantly different at $p \geq 0.05$ DMRT. The right cotyledon and left cotyledon results were treated separately.

+ callusing