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Multiple Shoot and Aerial Roots Induced from Various Explants of *P. tetragonolobus* L. (Winged Bean)

D.S.R. Naik*, T.N. Swamy and A. Seetaram Naik

Department of Botany, Kakatiya University, Warangal, Telangana State, India

*Corresponding Author E-mail: ravinaik0805@gmail.com

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ABSTRACT

Multiple shoots and aerial roots were directly regenerated in leaf and stem explants cultured on MS+BAP+IAA, BAP+NAA alone or in combination. MS+BAP (2.0 mg/L)+IAA (0.5 mg/L) respectively induced maximum number of multiple shoots with green callus, after four weeks of culture. This culture was transferred on ½ MS medium supplemented with BAP (2.0 mg/L)+IAA (0.5 mg/L) produced maximum number of multiple shoots and aerial roots from leaf explants, after eight weeks of culture. MS media supplemented with BAP (2.0 mg/L)+NAA (0.5 mg/L), induced adventitious roots with callus. These cultures were transfer on the media produced tuberous roots.

Key words: *Winged Bean, Explants, Leaf, Stem*

INTRODUCTION

Winged bean belongs to Papilionaceae family. It is grown as a vine with climbing, annual, perennial and herbaceous. One of the important aspects of world's nutritional problem concerns due to insufficient quantity of essential amino acids. Thirty (30) plant species provides 95% of world's food energy and 50% of the requirement for protein and calories[4]. But there are many underutilized Papilionaceae family plants that can make important contributions to the nutrition and health of people in developing countries. The leguminosae species includes very important vegetable and tuber crops[11] due to its high rich in protein content in tubers as well as in seeds, winged bean is an underutilized leguminous crop. Every parts of the winged bean provide a source of vitamin A and C, calcium, iron and other vitamins. It have been produces large root tubers (edible), which are rich in protein and are widely used as food (steamed, boiled and roasted) in tropical areas[17].

The Seeds of winged bean contain 23.9 to 42 % carbohydrates as well as tuber contains 27.2 to 30.5 % carbohydrates that provide energy. This infrequent combination makes the winged bean tuber unusual among the very hot and humid root crops[1].

As legumes, winged beans fix nitrogen, characterized by the ability to live in symbiotic relationship with a wide range of tropical *Rhizobium*[5,8]. They actually improve soil they grow in and their parts are particularly helpful in a compost pile.

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However variation in the effectiveness of different *Rhizobium* species and also in nodulation and nitrogen fixation among different selection of winged bean has been documented[7,9,10]. The high nodulation and nitrogen fixing rates have probably contributed to the exceptionally high level of protein in the various parts of the plant. Is usually considered to be recalcitrant to tissue culture techniques, there are some reports on successful tissue culture protocols in winged bean. In the present study multiple shoots, aerial roots and tuberous roots observed from the leaf and stem explants of winged bean.

MATERIALS AND METHODS

P. tetragonolobus dry Seeds variety NS 122 were procured from Nature Seeds Store, Malaysia, were surface sterilized with 70% ethanol for 3 minute, followed by treatment with 0.2% mercuric chloride for 4 minutes. They were rinsed thoroughly in sterile double distilled water then placed on MS medium. The seeds were germinated on MS basal media (Murashige and Skoog 1962) containing 0.8% agar under a 16h photoperiod at 25±1° C. All media were adjusted to p^H 5.6 - 5.8 before autoclaving at 121°C for 15 minute. Seed germinated within one week and after 10 days, the stem and leaf explants were excised from the seedlings, cut transversely into 1-2 cm long sections and used as explants.

RESULTS AND DISCUSSION

In our investigation to develop a protocol for multiple shoots and aerial roots formation from leaf and stem explants of *P. tetragonolobus* cultured on MS medium supplemented with BAP alone or in combination IAA or NAA.

Initiation of multiple shoots in both explants in most of the treatments ranged within four weeks of culture (Table-1). Rapid and early multiple shoots initiation were observed in concentrations of BAP (2.0 mg/L), when we applied the higher concentration of BAP it gives poor response. The present study, 64% of leaf and 52% of stem (fig. A) explants produced multiple shoots, where the maximum number of shoots was 6.24±0.13 per explants in leaf and 5.21±0.12 in stem, after four weeks of culture respectively (Table-1).

The multiple shoots regeneration were observed in MS+BAP+IAA (2.0 mg/L)+(0.5 mg/L). The present study, 88% of leaf and 84% of stem (fig. B) explants produced multiple shoots, where the maximum number of shoots was 13.54±0.31 per explants in leaf and 9.24±0.31in stem, after six weeks of culture respectively (Table-1).

The multiple shoots regeneration were observed in ½ strength MS+BAP+IAA (2.0 mg/L)+(0.5 mg/L). The present study, 92% of leaf and 88% of stem (fig. C) explants produced multiple shoots, where the maximum number of shoots and aerial roots was 18.16±0.28 and 11.42±0.31 per explants in leaf and 15.23±0.21 and 9.22±0.22 in stem, after eight weeks of culture respectively (Table-1).

The present study the adventitious roots were observed in combination of MS+BAP+NAA (2.0 mg/L)+(0.5 mg/L). The present study, 76% of leaf (fig. D) and 72% of stem (fig. E) explants induced maximum number of adventitious roots were 20 per explants in leaf and 12 in stem, after four weeks of culture. This culture was transferred on MS+BAP (2.0 mg/L) produced tuberous roots (fig. F) (Table-1)

Concentration of BAP also influenced the multiple shoot regeneration. At lower and higher concentration of BAP poor result was observed, while the concentration of BAP (2.0 mg/L) produced maximum number of multiple shoots. Barik *et al.*,[2]; Odutayo *et al.*,[13]; Tyagi *et al.*,[15], reported the importance of BAP in multiple shoot formation in chickpea and other legumes. The results of the presents study revealed that among the two explants used, leaf induced higher number of multiple shoots and aerial roots as compared with stem.

To fulfill the increasing requirement of nutrients and space the directly regenerated shoots from both explants were transferred in fresh medium in 500 ml conical flasks. Regenerated shoots started with development of the secondary lateral shoots and aerial roots. The initiation of multiple cultures on full and

½ strength MS media with various concentrations of BAP and IAA. The aerial rooting response was poor in full strength MS medium containing BAP+IAA (2.0 mg/L)+(0.5 mg/L), while in ½ strength MS media containing BAP+IAA (2.0 mg/L)+(0.5 mg/L), the maximum number of aerial roots were observed. Tran *et. al*.,[14], reported complete plantlet regeneration from either directly from the explants or through a callus phase in winged bean.

Similar result was reported in winged bean leaf explants on MS+NAA+BAP of *P. tetragonolobus* by Gregory *et al.*,[6]. Mehta and Mohan Ram[12] reported direct and indirect shoot regeneration in cotyledon and epicotyls explants cultured on MS+BAP (5×10^{-6}) in *P. tetragonolobus*. Bottino *et. al*.,[3], Venkateswaran *et al.*,[16] reported multiple shoots regeneration from epicotyls explants of *P. tetragonolobus*.

Table-1: Effect of different concentration of BAP, IAA and NAA on multiple shoot induction from leaf and stem explants of *P. tetragonolobu*s

Growth regulators	**Leaf explants**			**Stem explants**		
BAP mg/L	**% of response**	**No. of multiple shoot**	**No. of aerial root**	**% of response**	**No. of multiple shoot**	**No. of aerial root**
0.5	16	1.34±0.22	-	20	1.21±0.32	-
1.0	24	3.25±0.13	-	24	2.33±0.43	-
1.5	36	3.15±0.21	-	32	4.25±0.21	-
2.0	**64**	**6.24±0.13**	-	**52**	**5.21±0.12**	-
2.5	40	4.53±0.23	-	44	4.53±0.12	-
3.0	32	2.12±0.34	-	24	3.42±0.26	-
BAP +IAA mg/L						
0.5+0.5	24	3.56±0.21	-	28	2.54±0.21	-
1.0+0.5	36	5.22±0.13	-	40	6.22±0.13	-
1.5+0.5	54	6.01±0.44	-	52	8.10±0.46	-
2.0+0.5	**88**	**13.54±0.31**	-	**84**	**9.24±0.31**	-
2.5+0.5	68	5.26±0.18	-	72	11.36±0.11	-
3.0+0.5	56	4.34±0.24	-	64	6.14±0.21	-
BAP + NAA mg/L						
0.5+0.5	24	Rhizogenesis	-	24	Rhizogenesis	-
1.0+0.5	36	Rhizogenesis	-	32	Rhizogenesis	-
1.5+0.5	48	Rhizogenesis	-	40	Rhizogenesis	-
2.0+0.5	**76**	Rhizogenesis	-	**72**	Rhizogenesis	-
2.5+0.5	60	Rhizogenesis	-	52	Rhizogenesis	-
3.0+0.5	52	Rhizogenesis	-	40	Rhizogenesis	-
½ MS + BAP +IAA mg/L						
0.5+0.5	24	5.21±0.12	2.21±0.33	32	6.23±0.13	3.22±0.13
1.0+0.5	40	7.01±0.24	4.13±0.23	44	8.21±0.44	4.23±0.21
1.5+0.5	64	9.22±0.11	6.23±0.11	68	11.21±0.31	7.23±0.14
2.0+0.5	**92**	**18.16±0.28**	**11.42±0.31**	**88**	**15.23±0.21**	**9.22±0.22**
2.5+0.5	72	12.31±0.22	5.23±0.21	76	13.32±0.23	6.21±0.22
3.0+0.5	60	7.12±0.23	3.22±0.23	56	9.22±0.14	4.24±0.23

Fig. 1: Induction of multiple shoots, aerial roots and tubers from various explants on MS medium with different hormones

A. Induction of multiple shoots on MS medium with BAP (2.0 mg/L)

B. Maximum number of multiple shoots were produced on MS medium with BAP (2.0 mg/L) + IAA (0.5 mg/L)

C. Efficient multiple shoots and aerial roots were developed on ½ strength MS medium with BAP (2.0 mg/L) + IAA (0.5 mg/L)

D. Adventitious roots were obtained from leaf explants on MS medium with BAP (2.0 mg/L) + NAA (0.5 mg/L)

E. Adventitious roots were obtained from stem explants on MS medium with BAP (2.0 mg/L) + NAA (0.5 mg/L)

F. Tuberous roots were formed from sub-cultured stem explants.

CONCLUSION

In our study the ½ strength MS medium with various concentrations of BAP and IAA has been produced maximum number of multiple shoots and aerial roots from leaf explants than stem explants. The efficient tuberous roots were observed in MS medium along with different concentrations of BAP and NAA.

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