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Plant Regeneration from Leaf and Hypocotyl Explants of *Glycine wightii* (W. and A.) Verdc. var *longicauda*

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Conditions for the initiation and proliferation of shoots from leaf and hypocotyl explants of a perennial soybean, *Glycine wightii* (W. and A.) Verdc. var *longicauda* have been described. Multiple shoots were obtained from leaf and hypocotyl explants of 3～4 days old aseptically grown seedlings on MS (Murashige and Shoog's medium) supplemented with auxin, cytokinin combination. 0.1 mg/*l* l-naphthaleneacetic acid (NAA) and 1 and 5 mg/*l* indolebutyric acid (IBA) alone produced shoot buds. A combination of 0.1 mg/*l* NAA and 5 mg/*l* 6-benzylaminopurine (BAP) produced the highest frequency of shoot formation. 2,4-D despite supporting callus growth had no effect on organogenesis.

KEY WORDS: *Glycine wightii* var *longicauda,* plant regeneration, hypocotyl, 1-Naphthalene-acetic acid, Indolebutyric acid.

Introduction

According to Borlaug (1975) legumes comprise species of major agronomic importance. The annual soybean, *Glycine max* (L.) Merr. is as per the reports of Hilderbrandt *et al.* (1986) among the world's most important sources of vegetable oil and protein. *In vitro* methodologies have been proposed by Roth *et al.* (1985) as a supplement to traditional soybean improvement techniques. Plant regeneration has been reported by several authors viz. Cristianson *et al.* (1983), Ranch *et al.* (1985), Wright *et al.* (1987) in soybean in the past. Vaughan and Hymowitz *et al.* (1983) indicated that the wild perennial *Glycine* species possess agronomically important traits that could be transferred to the annual species by either classical plant breeding or genetic engineering techniques so as to alter soybean oil and protein quality as well as to enhance insect, viral and herbicide resistance. Regeneration via organogenesis in wild relatives of soybean has been reported for *G. canescens* by Kameya and Widholm (1981), Widholm and Rick (1983), Grant (1984), Hammatt *et al.* (1987) and for *G. clandestina* by Hammatt *et al.* (1986), Hymowitz *et al.* (1986), *G. falcata* and *G. latrobeana* by Hammatt *et al.* (1987) and *G, tomentella* by Kameya and Widholm, (1981), Hammatt *et al.* (1987). However, no attempt seems to have been made for *in vitro* regeneration of an important perennial species *G. wightii* (W. and A.) Verdc. syn. *G. javanica* auctt. mult. non L. used for soil conservation and as a cover crop. In the present study we report the *in vitro* regeneration of a wild perennial *Glycine* species *i.e., G. wightii* (W. and A.) Verdc var *longicauda.*

Material and Methods

Glycine (*Glycine wightii* (W. and A.) Verdc. var *longicauda*) seeds obtained from Royal Botanical Gardens, Kew, Richmond, Surrey (England) were surface sterilized with 0.1% mer-

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curic chloride (W/V) for 5 min and then rinsed in sterile distilled water 4～5 times. The seeds were germinated on sterile moist filter paper in petriplates/sterile moist cotton in flasks at 22～55°C in dark.

Hypocotyl and leaf explants were excised from 3 to 4 days old seedlings and placed aseptically on a solidified MS medium containing 3% sucrose and 0.8% agar. A total of 48 hypocotyl and 48 leaf explants were cultured on the media. The pH of the media was adjusted to 5.6～5.8 with 0.1 N sodium hydroxide and/or 0.1 N hydrochloric acid prior to adding the agar. The media were supplemented with filter sterilized auxins 1-naphthaleneacetic acid (NAA), indolebutyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D) and cytokinin 6-benzylaminopurine (BAP) both individually and in combination at a concentration of 0.1～10 mg/*l*. Cultures were maintained under white fluorescent light (40～50 uEM^{-2}sec^{-1}) with light/dark cycles of 16th/8h at 25 ± 2°C.

For cytological investigations 7～10 days old calli and 4～6 days old regenerated roots obtained on MS medium supplemented with various concentrations of auxin and cytokinin were pretreated with p-dichlorobenzene for 4～6 h, fixed with acetic acid : alcohol (1 : 3), hydrolysed with 1N hydrochloric acid for 10～15 min at 58～60°C and finally stained with 2% aceto orcein : 1N hydrochloric acid (9 : 1) as described earlier by SHARMA and SHARMA (1980). Chromosome analysis in the squash preparations was based on observation of 5 squashes in each concentration and an approximately 450～500 cells in each sample.

Results and Discussions

The two explants tested were found to be equally suitable for callus formation. Of the three auxins used, IBA was in general observed to be more suitable than 2,4-D or NAA for initiating callus. The best callus formation was observed in leaf explants with 1 mg/*l*, 2,4-D and callus formation along with rooting in leaf explants with 5 mg/*l* IBA. Callus formation in both the explants were rather a slow process taking two to three weeks period. Callus produced in the two explants with different concentrations of auxins and auxins and BAP showed variation in colour and texture. Callus obtained from leaf explants with different concentrations of auxins (NAA, IBA, 2,4-D) alone and in combination with BAP was albino and green in colour and soft and friable in texture. On the other hand callus derived from hypocotyl with different concentrations of auxins (NAA, IBA, 2,4-D) alone and in combination with BAP differed being light brown in colour and soft or hard and friable in texture. 0.1 mg/*l* NAA and 1 mg/*l* and 5 mg/*l* IBA alone induced shoot organogenesis in the explant (hypocotyl). However, the best shoot organogenesis was obtained in hypocotyl explants with 0.1 mg/*l* NAA and 5 mg/*l* BAP, 5～7 weeks after culture initiation. Whole plant formation was obtained in the same medium i.e. 0.1 mg/*l* NAA and 5 mg/*l* BAP after about 9 weeks in culture.

2,4-D alone or in combination with BAP though supporting callus growth has been found to be antagonizing organogenesis in the present study as reported in the past by RAO *et al.* (1973), MURASHIGE (1974), GROENEWALD *et al.* (1977), HUSSEY (1983) and LUPI *et al.* (1985). The occurrence of shoot differentiation with auxins (NAA and IBA) alone indicates that shoot regeneration is not essentially a function of cytokinin activity. This kind of auxin induced

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Table 1. Morphogenetic response of hypocotyl (H) and leaf (L) explans of *Glycine wightii* (W. and A.) VERDC. var *longicauda* after 60 days of incubation.

Growth media[1)]	Explant	Nature of response C R Sh	% Explant producing calli	% Explant producing roots	% Explant producing shoots
0.1 NAA	L	C — —	16.6	—	—
	H	C R Sh	25	25	25
0.5 NAA	L	C — —	50	—	—
	H	C — —	33.3	—	—
1 NAA	L	C — —	40	—	—
	H	C — —	33.3	—	—
5 NAA	L	C R —	85.7	28	—
	H	C — —	33.3	—	—
10 NAA	L	C R —	25	25	—
	H	C — —	66.6	—	—
1 IBA	L	C — —	50	—	—
	H	C — Sh	50	—	50
5 IBA	L	C R —	66.6	33.3	—
	H	C — Sh	100	—	50
10 IBA	L	C R —	33.3	33.3	—
	H	C — —	50	—	—
0.5 2,4-D	L	C — —	33.3	—	—
	H	C — —	50	—	—
1 2,4-D	L	C — —	100	—	—
	H	C — —	66.6	—	—
5 2,4-D	L	C — —	66.6	—	—
	H	C — —	25	—	—
0.1 NAA + 5 BAP	L	C — —	100	—	—
	H	C — Sh	100	—	100
0.5 NAA + 5 BAP	L	C — —	25	—	—
	H	C — —	33.3	—	—
1 NAA + 5 BAP	L	C — —	60	—	—
	H	C — Sh	50	—	50
1 NAA + 10 BAP	L	C — —	50	—	—
	H	C — —	50	—	—
5 NAA + 10 BAP	L	C — —	40	—	—
	H	C — —	25	—	—
0.5 2,4-D + 5 BAP	L	C — —	100	—	—
	H	C — —	100	—	—
5 2,4-D + 5 BAP	L	C — —	100	—	—
	H	C — —	100	—	—

C = Callus, R = Root, Sh = Shoot bud or shoot.
1) = Concentration of growth substances are expressed as mg/*l*.
— = No response.

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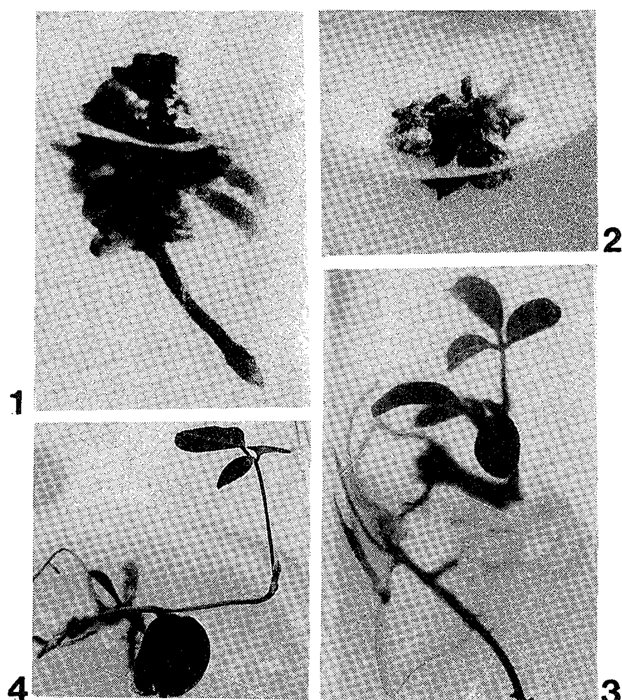

Fig. 1. **1** Leaf derived callus with regenerated roots on MS medium with 5 mg/*l* IBA. **2** Shoot bud initiation in hypocotyl derived callus on MS medium with 0.1mg/*l* NAA. **3**～**4** Whole plant formation in hypocotyl derived callus on MS medium with 0.1 mg/*l* NAA and 5 mg/*l* BAP.

shoot organogenesis has been reported in the past by DATTA and DATTA (1983).

Rhizogenesis of *G. wightii* was obtained along with shoot bud development with 0.1 mg/*l* NAA alone. But a suitable procedure for getting higher frequency of roots in the regenerated shoots needs to be developed.

Cytology of calli and regenerated roots revealed the existence of normal diploid (2n = 22) cells indicating the lack of chromosomal variability in callus as well as roots and establishing that organogenesis in *G. wightii* essentially occured at diploid level of genome.

Since sexual hybridisation between perennial species such as *G. wightii* and *G. max* has not been much successful as reported by HAMMATT *et al.* (1987), an alternative approach would be the use of protoplast fusion to produce somatic hybrids.

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Glycine wightii (W. and A.) VERDC. var *longicauda* の葉及び胚軸外植片からの植物体再生

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多年生のグリシン属野性種はダイズの油脂及びタンパク質の質的改変や耐虫性，耐病性のための遺伝子源を保有している.

これまでに *G. canescens*, *G. clandestina*, *G. falcata*, *G. latrobeana*, *G. tomentella* の器官形成を経た植物体再生の報告があるが *G. wightii* についての報告がない．この論文では *G. wightti* var*longicauda* を用いて，発芽後3～4日の葉及び胚軸外植片からの器官形成の条件を検討した．外植片を3％蔗糖，0.8％アガロースを含むMS培地に置床した．3種類のオーキシン（NAA, 1BA, 2, 4-D）を単独で，またはサイトカイニン（BAP）と組み合わせて0.1～10 mg/*l* の濃度で培地に加えた．培養は白色蛍光燈（40～50uEm^{-2} sec^{-1}）の下で16時間日長，25±2℃で行った．

胚軸外植片を用いて0.1mg/*l* の NAA と5mg/*l* の BAP で培養するとカルス形成後5～7週間にシュート形成率が最高であった．この条件で約9週間後に植物体が再生した．一方2, 4-D 単独又は2, 4-D と BAP との組み合わせではカルスは増殖するが，器官形成が認められなかった．シュートと根が同時に形成されたのは0.1mg/*l* の NAA のみを与えた場合だけであった．この実験で得られたカルスや再生した根の細胞は正常な2倍体であった．

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