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High frequency direct plant regeneration from petiole and leaf of Withania somnifera (L.)Dunal.

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Objectives

The present investigation was carried out to increase the efficiency and speed of regeneration from leaf, petiole and root explants and to compare their responses to different hormone concentration, media and culture conditions. We report an efficient and high frequency multiple, direct shoot regeneration from petioles and leaf explants that can be used for genetic transformation of Withania somnifera.

Materials and Methods

Seeds of Withania somnifera were surface-sterilization by 70% ethyl alcohol for 1 minute and subsequently soaked in a solution of 5 % (v/v) sodium hypochlorite for 15 minutes and washed with sterile distilled water. Sterilized seeds were planted in MS salt and incubated at 25-27 ℃ in 16 h photoperiod. After germination of seeds, plantlets were maintained in 1/2 MS medium.

Results

High frequency direct regeneration of shoots from petiole and leaf explants Withaniasomnifera (L.) Dunal has been developed. The shoots were mainly induced in the basal parts of leaf and petiole explants on Murashige and Skoog medium containing 0.1 mg/l naphthalene acetic acid (NAA), 2 mg/l 6-benzylaminopurine (BAP). It was observed that there was a considerable increase in shoot number on lower NAA (0.1 mg/l) whenever added to the medium in combination with TDZ (2.0 mg/l) or BA (2.0 mg/l) while higher levels of NAA lead to decline in shoot number. Histological investigations of regenerating shoot clearly showed that the shoot buds had emerged from the subepidermal parenchyma cells without any intermediate callus formation. Addition of 1 mg/l IBA to the medium was the most effective to induce root on which 100% of regenerated. The plants appeared normal and morphology of the plantlets was uniform to the seed derived mother plants.

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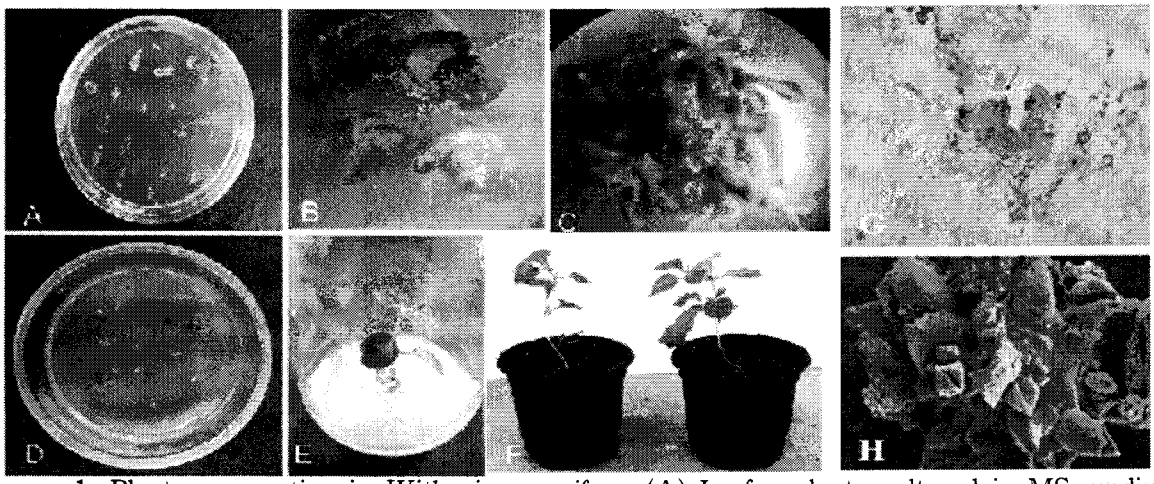

Figure 1. Plant regeneration in *Withania somnifera*. (A) Leaf explants cultured in MS medium supplimented with 2 mg/l BAP; (B) Direct shoot regeneration; (C) Multiple shoot; (D) Elongation of shoot; (E) Rooting of regenerated shoot. (F) Potted plants in the green house. (G) Longitudinal section of early regenerated shoots. (H) Scanning electron microscopic observations of shoot regeneration in *Withania somnifera* .

Table 1. The effect of plant growth regulators on callus induction and shoot regeneration from leaf and petiole on *Withania somnifera*.

Growth regulator (mg/l)				No of	% of	No. of root/explant	No. of	Callus
2,4-D	NAA	BAP	TDZ	explant	response		shoot/explant	Types
0.1	-	-	-	30	30	0	0	WF
1.0	-	-	-	30	80	0	0	WF
2.0	-	-	-	30	80	0	0	WF
3.0	-	-	-	30	100	0	0	WF
	-	1.0	-	30	76.67	0	0	GC
	-	2.0	-	30	83.33	0	23 ± 2.94	GC
	0.1	0.1	-	30	100	0.33 ± 0.47	0	GC
	0.1	1.0	-	30	100	0	9.67 ± 3.86	GC
	0.1	2.0	-	30	100	0	10.67 ± 3.40	GC
	1.0	0.1	-	30	100	7.67 ± 2.62	0	GC
	1.0	1.0	-	30	93.33	3.00 ± 1.63	0	GC
	1.0	2.0	-	30	96.67	0	0	GC
	2.0	0.1	-	30	100	12.00 ± 2.45	0	GC
	2.0	1.0	-	30	80	0	0	GC
	2.0	2.0	-	30	100	1.33 ± 0.47	0	GC
	0.1	-	0.1	30	76.67	0	1.33 ± 0.47	GC
	0.1	-	1.0	30	93.33	0	12.67 ± 2.06	GC
	0.1	-	2.0	30	100	0	16.67 ± 2.87	GC
	1.0	-	0.1	30	90	3.67 ± 1.70	0	GC
	1.0	-	1.0	30	100	0	0	GC
	1.0	-	2.0	30	93.33	0	0	GC
	2.0	-	0.1	30	83.33	0	0	GC
	2.0	-	1.0	30	83.33	0	0	GC
	2.0	-	2.0	30	100	0	0	GC

WF- White and Friable; GC- Green and Compact.