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PRELIMINARY STUDIES IN VITRO REGENERATION OF VETIVERIA ZIZANIOIDES THROUGH MERISTEM TIP CULTURE

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ABSTRACT

Vetiveria zizanioides is known as a vision plant because it possesses many valuable characteristics like erosion control, phytoremediation, medicinal properties etc. Vetiver is generally known to be originated in India. The present investigation was aimed to enhance the regeneration of Vetiver through meristem tip culture using Murashige and Skoog's (MS) medium supplemented with different types (1/2 MS & 1/4 MS medium) and concentration of plant growth regulators Kin (0.1mg/l), GA_3 (0.25mg/l), NAA (0.5mg/l) and BAP (0.25mg/l). It was found that the regeneration of Vetiver stimulates the growth in ¼ MS medium compare to ½ MS supplemented with Kin, GA_3, and BAP. However, the new numbers of shoots were noted at 7 days of interval for four weeks after sub culturing 2 times with same concentration of medium. The establishment of *Vetiveria zizanioides* through meristem tip culture is profligate growing for regeneration system. This procedure will be useful for imminent research in micropropagation and genetic transformation of *Vetiveria zizanioides*.

Keywords: *Vetiveria zizanioides, Meristem tip, BAP, GA_3, Kin, MS medium.*

No. of Tables: 1 **No. of References: 13**

INTRODUCTION

Vetiver (*Vetiveria zizanioides* L. Nash) a graminaceous plant seems to have originated in the area from India to Vietnam. It has been widely used for soil and water management. It's belonging to the family Poaceae. Commonly known as Khash-Khas, Khas or khus grass in India. Historically vetiver grass was well known in tropical countries for its aromatic and medicinal properties (Rao and Sussela, 1998). Medicinally, it is valued as a carminative, diaphoretic, ulcer, snake bite, scorpion sting, and also as a headache (Jaain, 1991). Another important use of Vetiver is in the essential oil industry. The viscous oil extracted from the roots has a sweet, woody and earthy character and is valued for its aroma and therapeutic properties (Massardo*et al*., 2006; Pripdeeveche*t a*l., 2006; Rotkittikhume*t al*., 2010).

Tissue culture techniques are used for the mass propagation of plant.Vetiver is propagated vegetatively by dividing its clumps into splits consisting of one or few shoots 15-20cm long including a portion of the roots (De guzman and Oyen, 1999). The splits start sprouting a week after transplanting, but growth is generally slow. Instead of using a large part of the mother clumps in vegetative propagation the tissue culture use only some special tissues of the Vetiver plant such as shoot tip, nodal buds or young inflorescences. Micropropagation via plant regeneration using stem sections (Han *et al*., 2004), axillary buds (Ma *et al*., 2006), leaves (Mucciarelli*et al*., 1993) and young inflorescences (Prasertsongskun, 2003) as explants has been reported.

The objective of this study was to develop a protocol for micropropagation through meristem tip explant using MS medium with different concentration and plant growth regulators.

MATERIALS AND METHODS

Plant Collection

Vetiveria zizanioides(Nash) roots were collected in Nehru Herbal Gardens from Nehru Arts and Science College, Coimbatore, Tamil Nadu, India. The identification was confirmed with Botanical Survey of India, Coimbatore, Tamil Nadu, India (Ref. No: BSI/SRC/5/23/2011-12/Tech-1673).

Medium used for experiment

For our *in vitro* experiments, MS (Murashige and Skoog 1962) of modified MS nutrient media supplemented with various concentrations of growth regulators, Sucrose (30%) and agar (0.8%) were used. The pH of the medium was adjusted to 5.8 with 1N NaOH and 1N HCL before autoclaving.

The modified MS medium contained ½ MS macronutrients, ½ MS micronutrients, 0.1 mg/l thiamine Hcl, 0.5 mg/l Pyridoxine Hcl, 0.5 mg/l Nicotinic acid, 100 mg/l Ferric EDTA and 2 mg/l Glycine were used with growth regulators Kin (0.1 mg/l), NAA (0.5 mg/l), GA_3 (0.25 mg/l), BAP (0.25 mg/l).

Establishment and maintenance of *in vitro* cultures

The explants of *Vetiveria zizanioides* were collected from the field. They were washed with sterile boiled water for 5 minutes. The explants were again washed thoroughly with washed with sterile water for 2 minutes. The roots were cut off and the outer leaf sheaths were removed. The explants were trimmed to

5 cm long from the above part. The explants were wiped with rectified spirit in sterile condition. Since the explants were transfer to air chamber remove the outer sheath and trimmed again to about 2cm long. Young whorls were taken and surface sterilized was done by using 0.1% mercuric chloride for 5 minutes respectively. Following the sterilization, the cuttings were put on modified MS medium supplemented with 30g/l sucrose, 0.8% agar, 0.1 mg/l kinetin, 0.5 mg/l NAA, 0.25 mg/l $GA_3$and 0.25 mg/l BAP. After inoculation, the tubes were incubated in the dark for 24 hours and were transferred to light and maintained under a 16/8 hour light/dark photoperiod at 25 ± 2^0C.

Statistical analysis:

Each treatment consisted of at least 20 explants and the experiment was repeated thrice. A complete randomized design was used in experiment. Analysis of variance and mean separation were carried out adopting Duncan's Multiple Range Test (DMRT) and the significance was determined at 5% level.

RESULTS AND DISCUSSION

The effect of various concentrations of growth hormones (GA_3, BAP, Kin and NAA) in meristem tip explants of Vetiver was studied. The different media used for direct regeneration from meristem tip explant is presented in (Table-1). The excised meristem tip explants from well grown plants were cultured on MS medium supplemented with various combinations and concentrations of BAP, NAA, Kin, and GA3. No regeneration response was recorded in the explant cultured on ¼ MS medium. Meristem tip (Fig.1) wascultured into the ½ and ¼ MS medium with different hormones. The initiation appeared after one week (Fig.2&3) in ¼ compared to ½ MS medium. As shown in Table-1 the elongation varied with ½ and ¼ MS medium (Fig.4&5). From the above combination ¼ MS medium obtained new number of shoot regeneration and elongation (Fig.6) with the measurement. The sub culture was done after 14 days of interval.

The main advantage of micropropagation is the production of many plants that are clones of each other. It is also used to produce disease free plants. Recently the multiple shoot regeneration is used to produce transgenic plant through genetic transformation in the recalcitrant varieties of economically important plants. In earlier study showed that liquid MS medium supplemented with 2 – 4 mg/l BAP of medium yielded the best multiplication in interval of six weeks (Le Van Be *et al*., 2008). Similarly Yang *et al*., 2013 shows best differentiation medium was MS + 1.0 mg/l BAP. The plant regeneration from callus culture of Vetiver on solid MS medium containing 0.45µM 2, 4-D shows after subsequent transfer to regeneration medium 65% of plantlets were obtained (Prasertsongskun, 2003). Based on this study, the solid ¼ MS medium supplemented with different hormones (Kin, Bap, NAA and GA_3) shows optimal for induction of shoot from meristem tip culture.

CONCLUSION

According to this finding, the system of plant regeneration from meristem tip culture of Vetiver in ¼ MS medium develop a new protocol for future research in genetic engineering and genetic transformation of *Vetiveria zizanioides*. This research has

shown that it is possible to reduce the cost of plantlet production during tissue culture. This can be achieved through the use of alternative sources of MS nutrients that are available locally. The low cost medium evaluated here can be adopted easily in the production of Vetiver planting material through meristem culture. This will greatly enhance availability of Vetiver planting materials at an affordable cost which will boost its production.

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***Table 1**: Effect of growth regulator formulation used for shoot induction from in vitro explant of Vetiveria zizanioides*

Culture medium	Concentration of growth hormone mg/L	Percentage of response
MS	0.25 mg/L BAP 0.25 mg/L GA_3 0.1 mg/L Kin 0.5 mg/L NAA	79.67± 2.52
½ MS	0.25 mg/L BAP 0.25 mg/L GA_3 0.1 mg/L Kin 0.5 mg/L NAA	80.33±3.51
¼ MS	0.25 mg/L BAP 0.25 mg/L GA_3 0.1 mg/L Kin 0.5 mg/L NAA	96.67±4.1

Results were expressed as mean ± standard deviation.

Fig 1

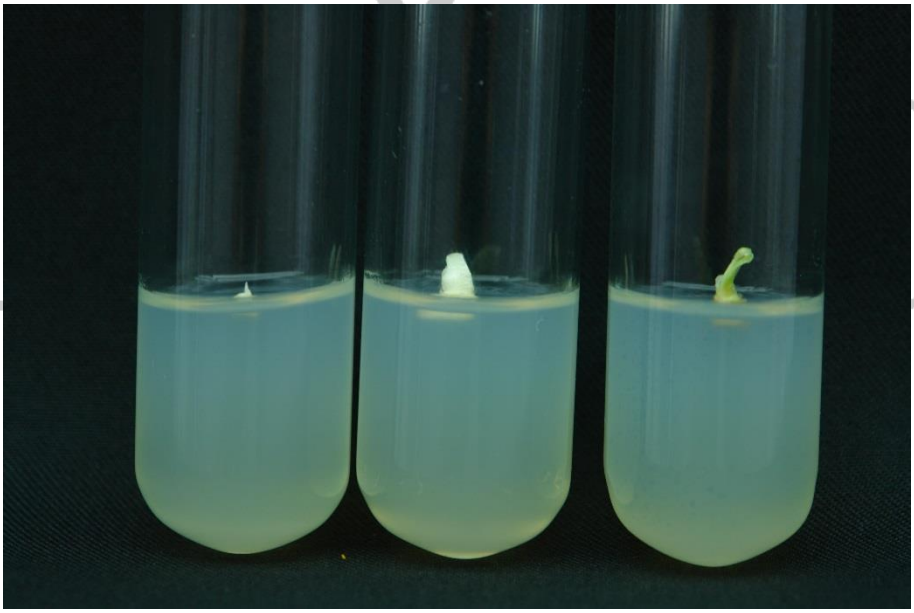

Fig 2

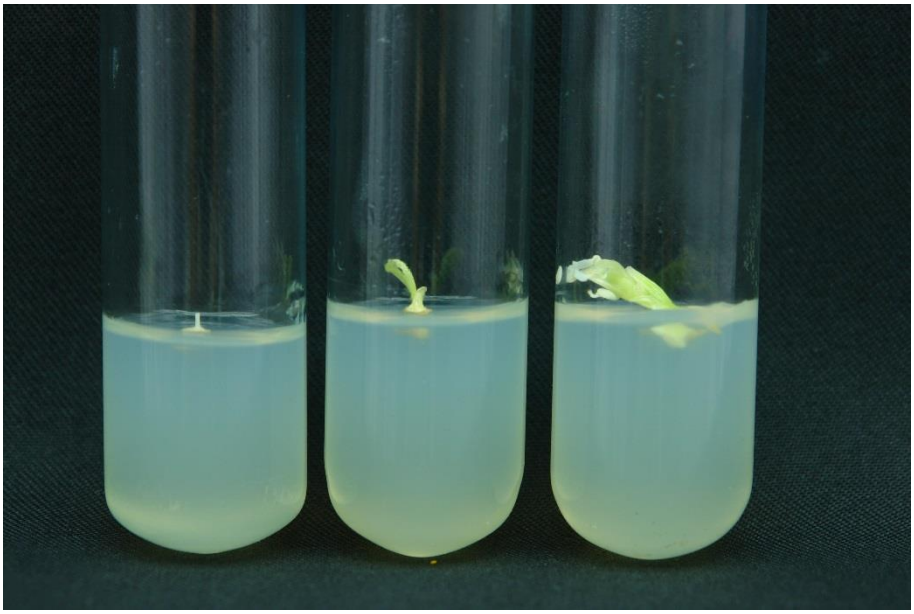

Fig 3

Fig 4

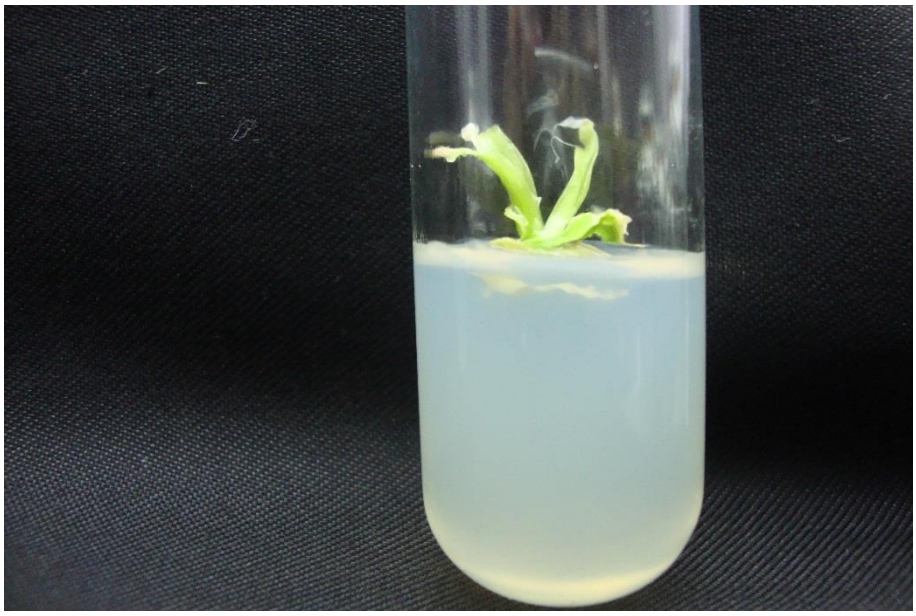

Fig 5

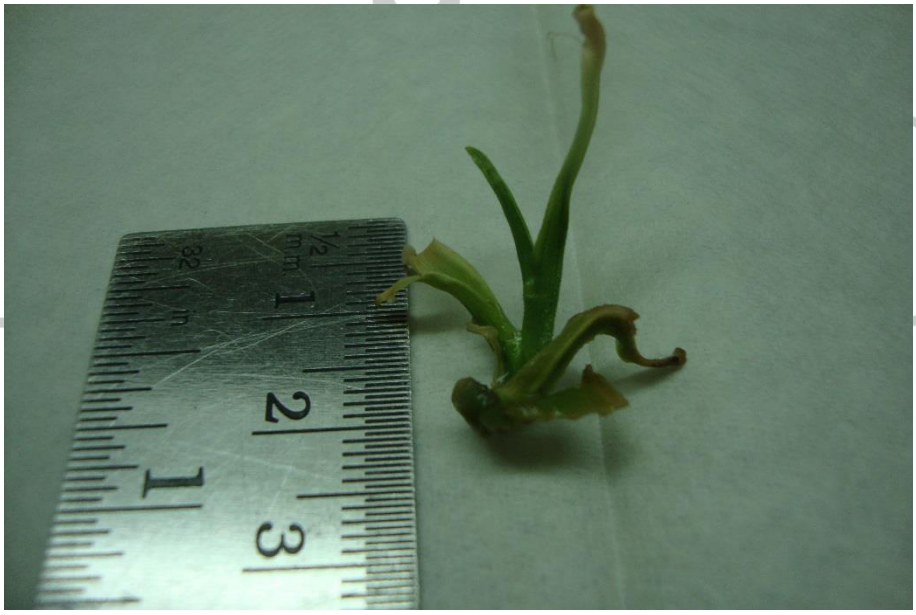

Fig 6

REFERENCES

De Guzman C., and Oyen L., *Vetiveria zizanioides* L.In Plant resources of south-East Asia.Essential-oil plants.191: 67-172, (1999).

Han J. S., Oh D. G., Mok I. G., Park H., Kim C. K., Efficient plant regeneration from cotyledon explants of bottle gourd (LagenariasicerariaStandl).*Plant Cell Reports*. 23: 291-296, (2004).

Jaain S. K., Dictionary of Indian folk medicine and EthnobotanyDeip Publi, New Delhi, (1991).

Le Van Be., Vo Thanh Tan., Nguyen Thi To Uyen., Le Viet Dung., Low-cost Micropropagation of *Vetiveria zizanioides*. *Assumption University Journal of Technology*. 12(1): 18-24, (2008).

Ma G. H., Xia H. P., XianY. L, Somatic embryogenesis and shoot formation from explants of *Vetiveria zizanioides.Journal of Tropical and Subtropical Botany*. 8: 55-59, (2000).

Massardo D.,Senatore F., Alifano P., Giudice L. D., Pontieri P., Vetiver oil production correlates with early root growth. *Biochemical Systematics and Ecoloy*. 34: 376-382, (2006).

Mucciarelli M., Gallino M., Scannerini S., Maffei M., Callus induction and plant regeneration in *Vetiveria zizanioides. Plant Cell, Tissue and Organ Culture*.3: 267- 71, (1993).

Murashigee T., and Skoog F., A revised medium for rapid growth and bioassays with tobacco tissue culture.*Journal of Plant Physiology*. 15: 473-497, (1996).

Prasertsongskun S., Plant regeneration from callus culture of Vetiver (*Vetiveria zizanioides*Nash). Songklanakarin.*Journal of Science and Technology*.25: 637- 42, (2003)

Pripdeevech P., Wongpornchai S., Promsiri A., Highly volatile constituents of *Vetiveria zizanioides* roots grown under different cultivation conditions. *Molecules*. 11: 817-826, (2006).

Rao R. R., and Suseela M. R., *Vetiveria zizanioides* (Linn.) Nash – A multipurpose ecofriendly grass of India. Paper presented at ICV-2 held in Cha-am, Phetchaburi, Thailand, 18-22 January 2000. In: Preceedings of ICV-2, 444-8, (1998).

Rotkittikhun P., Kruatrachue M., Pokethitiyook P., Baker A. J. M., Tolerance and accumulation of lead in *Vetiveria zizanioides* and its effect on oil production.*Journal of Environmental Biology*.31: 329-334, (2010)

Yang Bing-bing., and XIA Han-pinghen-rong., Study on tissue culture of *Vetiveria zizanioides*. *Acta Prataculturae Sinica*.2004-07, (2013).