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Direct adventitious shoot regeneration in *Piper longum* L. from spike explants

Vishal Prajapati, MM Patel, Suman Kumar Jha and Kalpesh Makwana

Abstract
Direct shoot regeneration was achieved from fruit spike explants of *Piper longum* L. Spike explants cultured on growth regulator-free basal MS medium showed no sign of bud break even after 30 days. Addition of a TDZ was essential to induce bud break and multiple shoot formation from the explants. The maximum response (26.0 per cent) observed in the media supplemented with 0.25 mg l^{-1} TDZ with average (16.80) number of shoot buds and (3.60) number of elongated shoot. Elongated shoots were carefully excised and rooted on previously established rooting media e.g. basal MS + 1.0 mg l^{-1} IBA, with 80 per cent rooting. Rooted explants successfully hardened in Soil: Sand: FYM (1:1:1) with 60% success.

Keywords: *Piper longum*, spike, direct regeneration, TDZ

Introduction
Traditional medicine is one of the most important ingredient of primary healthcare system. Either as active principal or as traditional formulation, in both ways medicinal plant has active role in combating disease from ancient time (Kumar and Van Staden 2016) [1]. The genus Piper belonging to family Piperaceae is source of several metabolites and essential oils of medicinal interest (Flores *et al.* 2009) [2]. *Piper longum* is one of the most important member of this genus in term of its medicinal properties. The population of *Piper longum* has decreased from the wild due to excessive extraction owing to its medicinal property. Traditional propagation through seed has problem like poor germination and heterogenous population whereas clonal propagation through cutting faces challenges like scanty and delayed rooting (Padhan 2015) [3]. These problems can be overcome by biotechnological interventions like *in vitro* regeneration. Some reports on micropropagation of *P. longum* are available (Bhat *et al.* 1995; Bhat *et al.* 1992; Parida and Dhal 2011; Rani and Dantu 2012; Soniya and Das 2002) [4-8], however, no report on direct regeneration through spike explants is available for the species. Hence, the present work was undertaken to see the potential of spike explants for large-scale propagation of *P longum* through direct regeneration pathways.

Material and Methods
Spikes were isolated from *Piper longum* L growing in green house at Forestry Nursery at College of Forestry, Navsari Agricultural University, Navasri. Spike explants were cultured on full strength MS media supplemented without any cytokinin or supplemented with various concentrations of TDZ (0.25,0.5,1.0,1.5 mgl^{-1}). Data on number of explants responding for shoot bud induction, average number of shoot bud per explants and number of elongated shoot recorded after four week of culture. Experiment was repeated thrice with 10 explants in each treatments. After harvesting elongated shoot, remaining clumps of shoot bud were cultured back in same media for further multiplication of shoots.
For rooting, microshoots (1.5–2.0 cm in length) were excised and transferred to half-strength MS medium supplemented with 1.0 mgl^{-1} IBA (previously established rooting media for *P. longum* in same laboratory). The rooted plants were removed from the culture bottles, washed free of agar with sterile distilled water and transferred to plastic pots with sterile Soil: Sand: FYM (1:1:1) media. The plantlets were maintained at 70% relative humidity by initially covering with transparent polyrthene. The plants were kept in 28^0C under a 12-h photoperiod for acclimatization. The plants were fertilized with 1/8th MS macro nutrients twice during the course of acclimatization at an interval of 4–5 wk.

The data recorded were analyzed for ANOVA (Analysis of Variance) for Completely Randomized Design (CRD). The mean were compared using critical difference at 5% significance level. All contaminated cultures were removed from the initiation experiments, thus limiting the scope of thorough statistical analysis. Wherever necessary, the data transformation (square root or angular) applied before analysis to normalize the data.

Results Discussion

In the present investigation, the morphogenetic response of spike explants to TDZ was evaluated. In our knowledge, this is first attempt of direct regeneration from spike explants in *P. longum*. Spike explants cultured on growth regulator-free basal MS medium showed no sign of bud break even after 30 days. Addition of a TDZ was essential to induce bud break and multiple shoot formation from the explants. Lower level of TDZ produced multiple shoot however, increasing the concentration of TDZ led to callus formation without any sign of bud differentiation. After 1 week culture on MS medium supplemented with various levels of TDZ, Organogenic response was observed in the form of small perturbation of green shoots bud. Thidiazuron (TDZ) is a cytokinin-like compound routinely used for *in vitro* culture studies including shoot proliferation and regeneration in various plants (Deepa *et al.* 2018) [9]. Distinguishable shoot buds cluster appeared in all responsive spike by the end of four weeks. Various stages of shoot bud development from spike explants are shown in Figure 1.

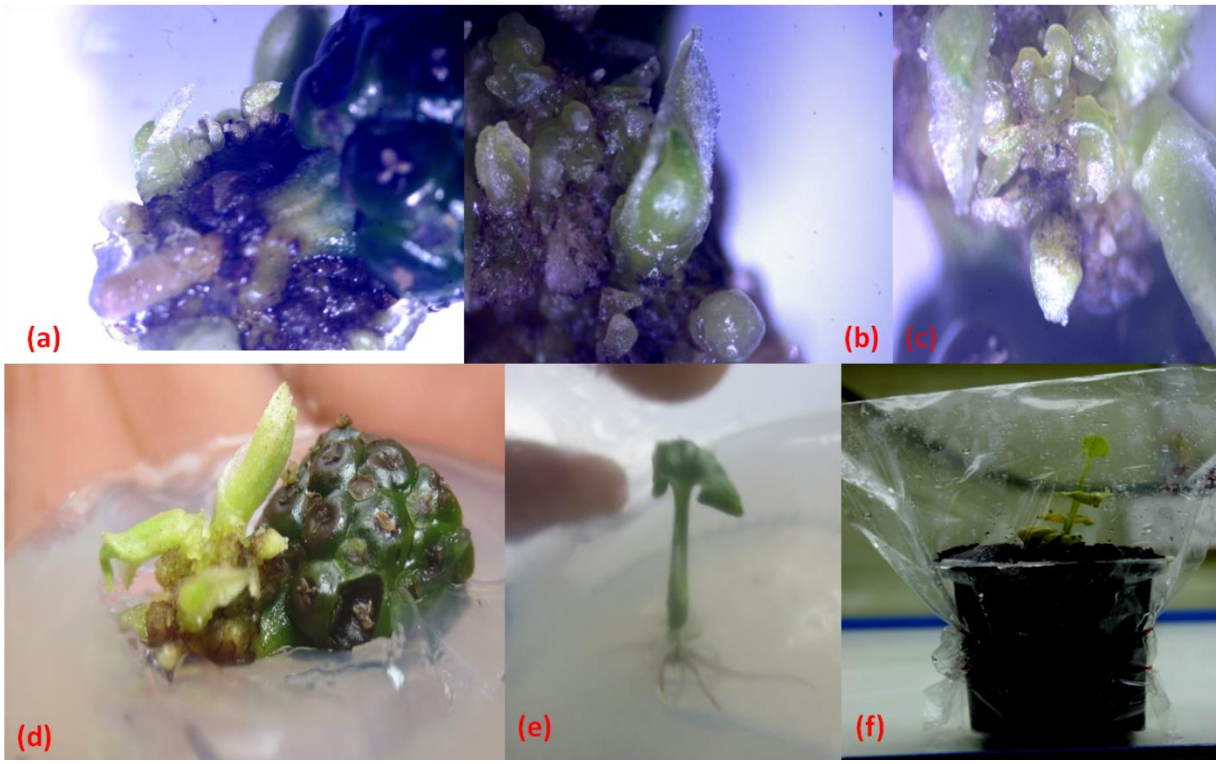

Fig 1: Direct organogenesis from spike explants in *P. longum* a) shoot bud initiation from spike explants b-c) shoot bud development d) shoot bud elongation e) rooting f) hardening of plantlets

The maximum response (26.0 per cent) observed in the media supplemented with 0.25 mgl^{-1} TDZ with average (16.80) number of shoot buds and (3.60) number of elongated shoots (Table 1).

Table 1: Effect of different concentrations of TDZ on direct shoot bud formation response (%), average number of shoot bud and average number of elongated shoot bud from fruit spike explants of *Piper longum* L. (Data recorded after four weeks of culture)

Treatments (mg/l)	Response (%)(##)	Number of shoot buds/spike (#)	Number of elongated shoots (#)
Control	0.00 (0.52)	0.00 (0.71)	0.00 (0.71)
0.25TDZ	26.00 (30.54)	16.80 (4.16)	3.60 (2.02)
0.5TDZ	24.00 (29.21)	15.00 (3.94)	2.40 (1.70)
1.0TDZ	14.00 (21.68)	8.60 (3.01)	1.40 (1.37)
1.5TDZ	12.00 (20.05)	8.20 (2.95)	1.00 (1.22)
S.Em±	1.54	0.06	0.06
CD at 5%	4.55	0.17	0.17
CV%	16.91	4.37	9.13

*Figures in prentheses are transformed values # square root transformation ## arcsine transformation.

TDZ successfully induced bud in *Lysima chialaxa* (Gupta *et al.* 2017) [10], *Jatropha curcas* (Kumar *et al.* 2018) [11] and *Chirita swinglei* (Chen *et al.* 2016) [12]. Frequency of shoot bud formation was not impressive and callus formation observed when higher concentration of TDZ added in media. The most common deleterious effect of higher concentration of TDZ are promotion in callus induction or reduction the shoot length (Malik *et al.* 2005; Parveen and Shahzad 2010; Ahmad and Anis 2007) [13-15]. Elongated shoots were carefully excised and rooted on previously established rooting media

e.g. Basal MS + 1.0 mgl^{-1} IBA, with 80 per cent rooting. Rooted explants successfully hardened in Soil: Sand: Vermiculite (1:1:1) with 60% success.

Conclusion

In conclusion, first time direct organogenesis from spike explants has been reported for *P. longum,* an important medicinal plant. Application of this protocol could help in conservation of plant species by minimizing the pressure on wild populations. In addition to this, the present system could permit genetic transformation studies for metabolic engineering in the near future.

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