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Turmeric, is an invaluable spice of immense economic importance in the world trade because of its use in culinary, religious, traditional and modern medicines for various human ailments. Turmeric is propagated vegetatively, since turmeric improvement programmes by conventional hybridization techniques have been hampered due to heterozygosity and lack of sexual cycle. Moreover, in vegetatively propagated plants like turmeric, it takes several years to evolve a superior clone by bringing together various important quality traits in a single genotype by breeding techniques. In addition, turmeric is highly susceptible to various diseases and pests, thereby resulting in considerable crop loss. Therefore, production and supply of disease free planting material to the farmers is very difficult. Under such circumstances, tissue culture techniques become more relevant in handling crop specific problems.

At present, most of the literature available on different varieties of Curcuma longa L. is concerned mainly on micropropagation. The present investigation includes development of repeatable protocols for direct multiplication, plant regeneration from callus phase through organogenesis and isolation of high yielding somaclonal variants.
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through callus phase. In addition, a protocol has been developed for isolation of root rot disease tolerant clones through continuous and discontinuous *in vitro* selection techniques using pure culture filtrate of *Pythium graminicolum*.

Further, in the present investigation the disease tolerance against pure culture filtrate of *P. graminicolum* in turmeric variety Suguna (*Curcuma longa* L.) was confirmed using a unique method of *in vitro* sick plot technique.

The major findings of the present study are summarized below.

- **LS basal medium** was found to be the best medium for shoot initiation and multiplication (1:8) from vegetative bud of turmeric variety Suguna (*Curcuma longa* L.) when compared to MS basal medium (1:2).

- **The in vivo** vegetative bud of turmeric variety Suguna produced more number of multiple shoots (36.25) on LSBM fortified with BAP (3.0 mg/l) when compared to Kn (16.58) with simultaneous development of root on the same medium. Further, this medium was found to be significantly superior with respect to multiple shoot formation. The axenic plants retrieved from the direct regeneration of non-embryogenic propagule *in vivo* vegetative bud were utilized as source material for induction of callus.

- **Two types of calli** were induced from non-embryogenic propagules *in vivo* vegetative bud, *in vitro* pseudostem, leaf, sheathing leaf base and root explants of turmeric variety Suguna: Compact creamish white callus on LS basal medium fortified with 2,4-D (2.0 - 3.5 mg/l) and loose friable brownish callus on LS basal medium fortified with NAA at different concentrations.
LS basal medium fortified with 2,4-D (2.0 - 3.5 mg l⁻¹) was found to be significantly superior in inducing large amount of compact creamish white callus when compared to LS basal medium supplemented with NAA.

It was found that the highest amount of callusing was observed from pseudostem with FW 9.86 g and DW 1.92 g (LSBM + 2, 4-D 2.5 mg l⁻¹) followed by leaf with FW 9.64 g and DW 1.77 g (LSBM + 2, 4-D 2.0 mg l⁻¹), vegetative bud with FW 9.41 g and DW 1.74 g (LSBM + 2, 4-D 3.0 mg l⁻¹), sheathing leaf base with FW 9.32 g and DW 1.71 g (LSBM + 2, 4-D 3.0 mg l⁻¹) and root with FW 9.14 g and DW 1.68 g (LSBM + 2, 4-D 3.5 mg l⁻¹).

On single passage of subculture of the calli, the compact creamish white callus on LS basal medium fortified with 2,4-D (2.0 - 3.5 mg l⁻¹) organized into morphogenetic callus. Whereas, the loose friable brownish callus on LSBM fortified with NAA failed to show morphogenetic response.

It was found that best organogenesis was induced from the compact creamish white callus obtained from in vitro leaf, pseudostem, in vivo vegetative bud, in vitro sheathing leaf base and root explants on LS basal medium supplemented with BAP 2.5 mg l⁻¹, 3.0 mg l⁻¹, 3.5 mg l⁻¹, 3.5 mg l⁻¹ and 4.0 mg l⁻¹ respectively. However it was observed that the differentiation of shoot buds was found to be more in leaf callus when compared to other calli formed from in vivo vegetative bud, in vitro pseudostem, sheathing leaf base and root explants. Large number of multiple shoots (31.23) was obtained from the leaf callus on the same medium. Further, it was found that complete plantlets were retrieved on the same medium thereby eliminating an additional step of rooting in vitro.

570 regenerants obtained through callus phase of in vivo vegetative bud (105), in vitro pseudostem (138), leaf (125), sheathing leaf base (110) and root (92) were screened for isolation of somaclones at culture conditions. In the
The present study, 19 somaclonal variants were identified and isolated which were classified into 9 types and grouped into 1-5 based on their origin and morphological traits.

The isolated somaclones and regenerants were hardened and established with 94% survival frequency using a potting mixture consisting of peat: perlite: vermiculite in the ratio 1: 1: 1 (v/v) with 90-95% Relative Humidity (RH) at 25 ± 2°C. These hardened plants were successfully established in field as (V1) generation (first generation following the in vitro phase).

Nine types of somaclones such as ‘Narrow elongated leaf with thick short pseudostem’ (evolved from the callus phase of vegetative bud and leaf), ‘Broad elongated leaf with very short pseudostem’ (evolved from the indirect regeneration of vegetative bud, pseudostem and sheathing leaf base), ‘Broad elongated leaf with thick short pseudostem’ (evolved from the callus phase of vegetative bud, pseudostem and root), ‘Broad short leaf with very short pseudostem’ (evolved from the callus phase of vegetative bud, pseudostem, leaf and sheathing leaf base), ‘Broad short leaf with normal pseudostem’ (evolved from the callus phase of vegetative bud, leaf and sheathing leaf base), ‘Broad elongated leaf with thick elongated pseudostem’ (evolved from the callus phase of pseudostem), ‘Broad short leaf with thick elongated pseudostem’ (evolved from the indirect regeneration of leaf), ‘Broad short leaf with thick short pseudostem’ (evolved from the callus phase of sheathing leaf base) and ‘Narrow short leaf with very short pseudostem’ (evolved from the indirect regeneration of root) were isolated based on the morphological characters such as plant height, number of tillers per clump, number of leaves per clump, leaf length, leaf breadth, yield of rhizomes per clump and dry recovery and classified into 1-5 groups.

The somaclones showing higher values of the metric traits than the regenerants and control with regard to morphological characters in the first
generation (V₁) were selected for further evaluation in the second generation (V₂).

"Narrow elongated leaf with thick short pseudostem" (Group 1: in vivo vegetative bud); "Broad elongated leaf with thick elongated pseudostem" (Group 2: in vitro pseudostem); "Broad short leaf with thick elongated pseudostem" (Group 3: in vitro leaf); "Broad short leaf with normal pseudostem" (Group 4: in vitro sheathing leaf base) and "Broad elongated leaf with thick short pseudostem" (Group 5: in vitro root) were found to be superior with regard to biochemical traits such as curcumin, oleoresin, volatile oil contents and the genomic DNA concentration when compared to other somaclones, regenerants and control Suguna.

For the first time, an attempt has been made to develop a repeatable protocol for isolation of somaclones which were superior to the regenerants and control through callus phase of different non-embryogenic vegetative propagules of turmeric variety Suguna (C. longa L.), which may open up new perspectives for the genetic improvement of turmeric varieties.

A repeatable protocol has been developed to isolate root rot disease tolerant clones of turmeric variety Suguna of C. longa using continuous and discontinuous in vitro selection techniques against pure culture filtrate of P. graminicolum.

It was observed that 2% pure culture filtrate of P. graminicolum was found to be the optimum concentration (LD₅₀/₃₀) to induce tolerance to callus (cells) when compared to other concentrations tried within 30 days of treatment.

Different cell lines (CL1a₁, CL1a₂, CL1b and CL2) were isolated after three cycles of continuous in vitro cell selection technique by treating pure culture
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Filtrate of *P. graminicolum* to the calli derived from different non-embryogenic propagules such as *in vivo* vegetative bud, *in vitro* pseudostem, leaf, sheathing leaf base and root explants.

The statistical analysis pertaining to fresh weight and dry weight of the callus revealed significant differences between control and cell lines CL1a₁, CL1a₂, CL1b and CL2 derived from three cycles.

The complete plantlets (2,064) were retrieved from the tolerant calli of different cell lines through discontinuous *in vitro* selection technique.

The tolerant clones were isolated after two cycles of continuous *in vitro* sick plot technique. The tolerant : susceptible ratio of the retrieved regenerants was found to be 1,112 : 241 (CL1a₂ – 298 : 122; CL1b – 374 : 84 and CL2 – 440 : 35). It was interesting to observe that all the plants retrieved from the non-selected and CL1a₁ calli did not survive (Control – 00 : 901; CL1a₁ – 00 : 711) under *in vitro* sick plot selection pressure.

In the present study, 82% of root rot disease tolerant clones were isolated after two cycles of continuous *in vitro* sick plot technique, which are maintained as separate root rot disease tolerant clones and established in soil with 90% survival frequency.

*In vitro* continuous-discontinuous selection and *in vitro* sick plot techniques will have a far-reaching implication for evolving disease tolerant clones in different economically important crops. These novel techniques will certainly replace the traditional breeding methods for evolving disease resistance, which lead to new tolerant clones with stable genetic background.