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IN VITRO PLANTLET REGENERATION FROM COTYLEDONARY EXPLANTS IN *SOLANUM TORVUM (*SWARTZ). A MEDICINALLY IMPORTANT PLANT

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ABSTRACT
In vitro culture of medicinal plants is important for the production of plant based high quality medicine. *Solanum torvum* (Swartz) possess immense medicinal importance and *in vitro* culture for commercial production was achieved by culturing the plant using different concentration and combination of plant growth regulators. Mainly, the concentration and combination of auxins and cytokinins in the nutrient MS medium is the key factor which determines successful plant regeneration. *In vitro* plantlet regeneration has been obtained from 15-20days old seedling cotyledon segments of *Solanum torvum* (Swartz) were examined using various phytoharmons individually and in combination on Murashige and Skoog (MS) semi solid medium supplemented with BAP/Kn/TDZ (1.0-5.0 mg/L) IAA (0.5 mg/L)+ BAP/Kn/TDZ (1.0-5.0 mg/L) and IAA (0.5 mg/L) for shoot proliferation IAA (0.5 mg/L)+BAP (3.0 mg/L) was proved to be best for induction of shoots for Cotyledon explants. Individual shoots were aseptically excised and sub cultured in the same media for shoot elongation. The elongated shoots were transferred to Indole Butyric Acid (IBA) (1.0mg/L–5.0mg/L) for root induction. Rooting was observed within two weeks of culture. Rooted plantlets were successfully hardened under culture conditions and subsequently established in the field conditions. The recorded survival rate of the plants was 86%. Plants looked healthy with no visually detectable phenotypic variations.

KEYWORDS: *Solanum torvum* (Swartz), Plantlet formation, *In vitro* rooting shoot formation.

Abbreviations: BAP, 6-Benzyl amino purine;; Kin, Kinetin TDZ Thioduzuron IAA, Indole acetic acid MS, Murashige and Skoog.

INTRODUCTION
The family *Solanaceae* is composed of approximately 90 genera and between 2,000 and 3,000 species. The family is widely distributed throughout tropical and temperate regions of the world, with center of diversity occurring in Central and South America and Australia. Within this family, Solanum is the biggest genus in the family, with the widest range of geographical distribution. In Solanum in particular, 41 species are represented in India, of which 19 are native, 8 naturalized, 10 cultivated and 4 cultivated experimentally, out of a total of 1700 species recorded all over the globe (Deb, 1979). *Solanum torvum* commonly known as turkey berry, devil's-fig, or prickly *Solanum* (Pier 2003) is a plant of great economic importance. Its extract, rich in Solanine and Solasodine (steroidal alkaloids) have beneficial effect on bronchial asthma. It is cultivated in tropics for its immature edible fruits (Langeland and Burks 1998). Alkaloids are a class of nitrogen compounds structurally diverse, found in all plant groups, curring most of them in angiosperms (Henriques *et al.*, 2002; Hughes and Shanks, 2002).

Many species of the Solanaceae have been regenerated by shoot organogenesis using young leaf explants. *Solanum surattense* (Gupta and Handra, 1982), *Solanum candidum*, *S.quitoense*, *Solanum sessiliflorum* (Hendrix *et al.*, 1987), *Solanum melongena* (Mukherjee *et al.*, 1991) and *Solanum commersonii* (Cardi *et al.*, 1993). Arulmozhi and Ramanujam (1997) conducted *in vitro* culture studies on *Solanum trilobatum* L. with foliar and stem explants on MS medium containing IAA, BAP and KIN combinations. Madhavan *et al.*, (1998) induced high frequency of shoot regeneration from mature seeds of *Solanum trilobatum* L. Callus was induced from root and shoot apical region and hypocotyls on MS medium supplemented with 2,4-D. The plant tissue culture methods also provide base for the improvement of crop. To induce somaclonal variations, *in vitro* mutations, herbicide tolerance, di-haploid induction, genetic transformation of economically important genes and development of somatic hybrids, efficient plant regeneration protocol is required. Such advance tech-niques in combination with conventional breeding give a

momentum to the improvement of a crop. Thus, realizing the prospects for future research, relevant literature to "Plant regeneration in *Solanum torvum* (Swartz)" has been studied.

METHODOLOGY

Plant material

Seeds of *S.torvum* were collected from Botanical garden Department of Botany SRR Govt. Degree & P.G. College Karimnagar. Dried mature seeds were soaked in Dilute Sulphuric acid (H_2SO_4) for 24 hrs and sterilized with 0.1% (w/v) aqueous Mercuric chloride ($HgCl_2$) for 3-5 minutes followed by washing 3 times with sterile distilled water later these were dried on sterile tissue paper under container air flow 20 seeds per culture bottle were germinated aseptically on MS basal medium containing 3% (w/v) sucrose and 0.8% (w/v) agar. These culture bottles were incubated at 25 ± 1ºC under16 h photoperiod. Light was provided by cool white fluorescent tubes with an intensity of 50-60 µ mol m^{-2} $s^{-1.}$

Data analysis

20 replicates were maintained for each treatment. Each treatment was repeated at least once with similar results. Data were recorded after eight weeks of culture.

RESULTS

The role of cytokinin and Auxin- cytokinin combinations on direct plant regeneration and adventitious bud induction from cotyledon hypocotyl and leaf explants was studied in order to find out the efficient protocol and potential on MS medium fortified with different concentrations of cytokinins alone and auxin's (0.5mg/L) in combination with various concentrations of cytokinins such as BAP/Kn/TDZ (1.0-5.0 mg/L). These explants were enlarged 3-4 fold within one week of culture initiation. Morphogenic change as were apparent after 6 weeks of culture. The leaf explants developed shoot primordial in large numbers directly from all cut surfaces in contact with medium in all the concentrations and combinations of phytohormones used even in combination with (0.5 mg/L) IAA. The results are presented in Table (1-2).

Cotyledonary explants

Cotyledonary explants cultured on MS medium containing various concentrations of cytokinins BAP/Kn/TDZ alone the direct shoot regeneration (Table-8) and (0.5 mg/L) IAA in combination with (1.0-5.0 mg/L) BAP/Kn/TDZ showed variable response in cotyledonary explants culture of *S.torvum.* These explants were elongated 3-4 folds within one week of culture initiation. Morphogenic changes were apparent after 6 weeks of culture. The results are presented in (Table 1-2) and shown in (Fig-1).

Effect of BAP

Cotyledonary explants were cultured on MS medium amended with various concentrations of BAP (1.0-5.0 mg/L) as role growth regulators showed the direct organogenesis (Table -1) Maximum number of shoot bud proliferation (26.5 ± 0.32 shoots /explant) was found at (3.0 mg/L) BAP. At 1.0, and 2.0 mg/L BAP induced (12.6 ± 0.42 and 18.3 ± 0.47) shoots/explant with 55 and 65% of cultures responded. When the concentration was increased up to (3.0 mg/L) BAP gradually induction of multiple shoots were reduced. At (4.0 and 5.0 mg/L) BAP induced (20.3 ± 0.13 and 10.0 ± 0.12) shoots/explants with 75 and 58 percentage of cultures were responded. The number of shoot bud induction was found to be decreased as the concentration of BAP increased. At high concentration of BAP showed less number of shoots per explants.

Table 1: Effect of BAP, Kn and TDZ on Direct shoot bud Proliferation of *Solanum torvum* (Swartz) from Cotyledonary explants.

Hormone concentration (mg/L)	% of cultures responding	Average No. of shoots / Explants ± (SE)*	Average length of shoots (cms) ± (SE)*
BAP			
1.0	55	12.6 ± 0.42	1.4 ± 0.52
2.0	65	18.3 ± 0.47	2.0 ± 0.16
3.0	82	26.5 ± 0.32	3.4 ± 0.14
4.0	75	20.3 ± 0.13	3.0 ± 0.12
5.0	58	10.0 ± 0.125	1.2 ± 0.05
Kn			
1.0	48	10.2 ± 0.12	1.3 ± 0.05
2.0	56	13.8 ± 0.19	2.3 ± 0.32
3.0	72	20.0 ± 0.12	2.8 ± 0.43
4.0	70	18.0 ± 0.14	2.3 ± 0.14
5.0	53	09.2 ± 0.13	1.8 ± 0.23
TDZ			
1.0	56	13.2 ± 0.15	1.2 ± 0.32
2.0	66	19.8 ± 0.12	2.2 ± 0.23
3.0	84	28.0 ± 0.35	2.4 ± 0.32
4.0	78	25.8 ± 0.34	2.2 ± 0.13
5.0	56	16.5 ± 0.35	1.4 ± 0.24

Mean ± Standard Error

Effect of Kn

Cotyledonary explants were cultured on MS medium containing different concentrations of cytokinin Kn (1.0-5.0 mg/L) as role growth regulators showed the direct organogenesis/ shoot formation (Table -1) to find out the difference between BAP and Kn in inducing the direct plant regeneration from cotyledonary explants in *S. torvum*. Maximum number of shoot bud proliferation (20.0 ± 0.12 shoots/ explant) was found at (3.0 mg/L) compared to all other concentrations of Kn at (1.0 and 2.0 mg/L) Induced (10.2 ± 0.12 and 13.8 ± 0.19 mg/L) shoots / explant with 48 and 56 percentage of cultures responded. Percentage of response was gradually increased up to (3.0 mg/L) Kn. At above (3.0 mg/L) concentration less number of shoots was recorded. At (4.0 and 5.0 mg/L) Kn induced (18.0 ± 0.14 and 9.2 ± 0.13) shoots/explants with 70 and 53 percentage of cultures were also recorded on MS + Kn. However high induction of ability was found in all the concentrations of Kn compared to BAP.

Effect of TDZ

TDZ was more responsive compared to BAP and Kn in inducing shoot buds from the explants (Table-1) with (1.0 mg/L) TDZ the cotyledon explants produced (13.2 ± 0.15 shoots/ explants and 56% cultures responded (3.0 mg/L) TDZ was more responsive in inducing maximum number of shoots (28.0 ± 0.35 shoots/ explants) with a greater frequency 84% TDZ at (4.0 and 5.0 mg/L) produced (25.8 ± 0.34 and 16.5 ± 0.35) shoots/ explants with 78% and 56% culture responding. As the concentrations of TDZ were increased from (3.0 mg/L to 5.0 mg/L) the number of shoots per explants was considerably reduced (Table-1).

Effect of IAA + BAP

To find out the influence of auxin-cytokinin combination on direct regeneration. The cotyledonary explants were cultured on MS medium fortified with (0.5 mg/L) IAA and different concentrations of cytokinin such as BAP (1.0 -5.0 mg/L). IAA in combination with various concentrations of BAP showed variable results (Table -2). Highest percentage of response was observed at (0.5 mg/L) IAA + (3.0 mg/L) BAP. The percentage of response and number of shoots proliferation was increased up to (1.0 mg/L) BAP and later gradually decreased at above (3.0 mg/L) BAP. At (1.0, 2.0 and 3.0 mg/L) BAP with IAA (0.5mg/L) induced (18.4± 0.12, 21.3± 0.23 and 28.2 ± 0.17) shoots/explants with 55, 62 and 86 percentage of cultures were recorded. At the concentrations of BAP was gradually increased above (3.0 mg/L) after wards decreased the number of shoots. (4.0 and 5.0 mg/L) BAP+ IAA (0.5 mg/L) induced (26.3± 0.12 and 16.0 ± 0.23) shoots/explants with 76 and 58 percentage of response was recorded. (Table 2).

Effect of IAA +Kn

Cotyledonary explants cultured on MS medium augmented with (0.5 mg/L) IAA and different concentrations of Kn (1.0 -5.0 mg/L) Showed variable response was recorded (Table -2). Maximum shoot bud induction was recorded at (3.0 mg/L) (25.0 ± 0.32 shoots/ explants) with 80 percentage of response was recorded. At (1.0 and 2.0 mg/L) Kn + (0.5 mg/L) IAA induced. (14.2 ± 0.12 and 20.4 ± 0.34 shoots/ explants) with 52 and 60 percentage of cultures were recorded. When the Kn concentration was increased from (3.0 to 5.0 mg/L) it was found that the shoot regeneration and percentage of responses was decreased. (Table -2).

Effect of IAA +TDZ

Cotyledonary explants cultured on MS medium augmented with IAA (0.5 mg/L) and different concentrations of TDZ (1.0 -5.0 mg/L) Showed variable response was recorded (Table -2). Maximum shoot bud proliferation was recorded at (3.0 mg/L) (16.5 ± 0.35 shoots/ explants) (Fig-b) with 87 percentage of response was recorded. At (1.0 and 2.0 mg/L) TDZ + (0.5 mg/L) IAA induced. (16.5 ± 0.35 and 23.0 ± 0.54 shoots/ explants) with 56 and 64 percentage of cultures were recorded. When the concentration of TDZ was increased from (3.0 to 5.0 mg/L) it was found that the shoot regeneration and percentage of responses was decreased (Table -2).

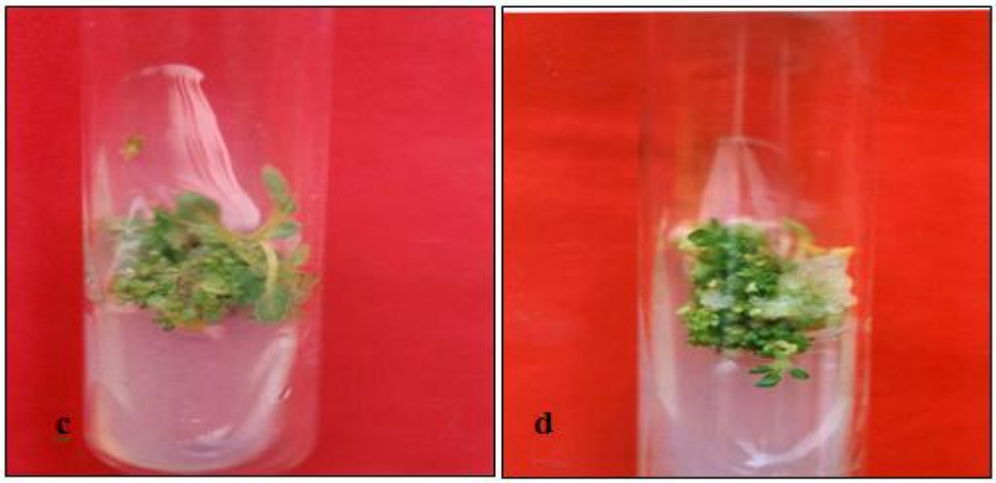

Figure1: *In Vitro* Plantlet regeneration from Cotyledonary explants in*Solanum torvum (*Swartz). a)*In Vitro* Plantlets on (3.0mg/L) BAP from cotyledon culture c) Multiple shoots on IAA(0.5mg/L)+(3.0mg/L) BAP from cotyledon culture d) *In Vitro*shoots formation on IAA(0.5mg/L)+BAP(3.0mg/L) after six week.

***In vitro* rooting**

Fully elongated healthy shoots were transferred on to full strength MS root induction medium (RIM) (Murashige and skoog 1962) fortified with different concentration of IAA (0.5 – 2.0 mg/L) and IBA (0.5 – 2.0 mg/L). Profuse rhizogenesis was observed on 1.5 mg/L IAA, compared to 0.5 -2.0 mg/L) IAA/ IBA on MS medium containing 1.5 mg/L IBA whereas 96% of plants produced roots with 14.3 ± 0.27 roots/ explant.

Acclimatization

Rooted plantlets were removed from the culture medium and the roots were washed under running tap water to remove agar. Then the plantlets were transferred to polypots containing pre- soaked vermiculite and maintained inside a growth chamber set at 28 ^{o} C and 70 – 80 % relative humidity. After three weeks they were transplanted to poly bags containing mixture of soil + s and + manure in 1: 1: 1 ratio and kept under shade house for a period of three weeks. The potted plantlets were irrigated with Hogland's soulation every 3 days for a period of 3 weeks.

Table 2: Effect of IAA in combination with BAP, Kn and TDZ on Direct shoot bud proliferation of *Solanum torvum* (Swartz) from Cotyledonary explants.

Hormone concentration (mg/L)	% of frequency of plantlet production	Average No. of shoots / Explants ± (SE)*	Average length of shoots (cms) ± (SE)*
IAA + BAP			
0.5 + 1.0	55	18.4 ± 0.12	2.4 ± 0.12
0.5 + 2.0	62	21.3 ± 0.23	2.6 ± 0.12
0.5 + 3.0	86	28.2 ± 0.17	3.4 ± 0.17
0.5 + 4.0	76	26.3 ± 0.12	3.2 ± 0.12
0.5 + 5.0	58	16.0 ± 0.23	2.3 ± 0.25
IAA + Kn			
0.5 + 1.0	52	14.2 ± 0.12	1.6 ± 0.35
0.5 + 2.0	60	20.4 ± 0.34	2.4 ± 0.13
0.5 + 3.0	80	25.0 ± 0.32	3.2 ± 0.34
0.5 + 4.0	72	23.8 ± 0.32	3.0 ± 0.24
0.5 + 5.0	56	14.2 ± 0.45	2.0 ± 0.34
IAA + TDZ			
0.5 + 1.0	56	16.5 ± 0.35	1.2± 0.32
0.5 + 2.0	64	23.0 ± 0.54	2.0 ± 0.32
0.5 + 3.0	87	29.5 ± 0.35	2.6 ± 0.42
0.5 + 4.0	78	24.0 ± 0.62	2.8 ± 0.42
0.5+ 5.0	59	16.5 ± 0.34	2.0 ± 0.46

* Mean ± Standard Error

DISCUSSION

We were successful in direct regenerating plants from cotyledonary and leaf explants of *S. torvum* cultures on MS medium fortified with different concentrations of cytokinins i.e. BAP/Kn/ TDZ (1.0-5.0 mg/L) individually and also in combination with (0.5 mg/L)

IAA. Maximum number of shoot buds was induced at (3.0 mg/L) TDZ in comparison to Kn/BAP as role growth regulators. When the low level of auxin (0.5 mg/L) IAA were added to the medium containing BAP/Kn/TDZ. It was interesting find out that the shoots induction was enhanced in all the concentrations of cytokinins. However the shoot bud proliferation was found to more on (0.5 mg/L) IAA in combination with TDZ compared to (0.5 mg/L) in combination with Kn/BAP but the combination of IAA+TDZ induced highest number of plantlet regeneration among all hormonal combinations and concentrations were used in *S. torvum*.

Similarly Hoque *et.al.,* (2000) have reported the high frequency of plant regeneration on Ms medium containing 2.0mg/L BAP in combination with 0.5 mg/L IAA from cotyledon derived callus in *Momordica dioica,* of the cytokinins used Kn proved as most effective than BAP in inducing shoots, the same findings were recorded in *Capsisum* Spp.(Venkataiah and Subhash 2002).

A protocol on plantlet regeneration through somatic embryogenesis from cotyledon and leaf explants and streptomycin resistant plantlets using *in vitro* mutagenesis was also developed in *S.surattense. Agrobacterium* mediated genetic transformation using leaf explants of *S.surattense* was also reported by Rama Swamy.[16]

The explants cotyledon hypocotyls and leaf cultured on MS medium supplemented with IAA+BAP combination was found to be more effective in inducing maximum number of shoots in *Capsicum* Spp (Gunay and Rao,1978), similarly it was also reported in *Capsicum annuum* (Christopher and Rajam,1996) and *Solanum melongena* cv Pusa round (Sharma and Rajam,1995). The combination of IAA+BAP showed superiority over IAA+Kn in all the explants. Similar finding were also made in *Petunia* (Rao *et,al.,*1973) *Lycoperiscon esculentum* (Kartha *et.al.,*1976) and *Solanum incanum* (Sadanandam and Farooqui,1997) and in two species of Niger (Jadimath *et.al,*1998).

Shakazad *et.al.,*(1999) have also observed the highest frequency of direct shoot regeneration on lower level of Auxin and high level of Cytokinin (4.0 mg/L) BAP+(0.5mg/L) IAA) in leaf explants of *S. nigrum*. Similar results were obtained in *Dalbergia Canceolats* (Dwari and Chand 1996). However on lower level of BAP the frequency of shoot regeneration was decreased similar to our present observations. Highest numbers of shoots per explant were developed on MS Medium containing (0.5mg/L) IAA+ (2.5mg/L) BAP in leaf explants of *Solanum sisymbrifolium* (Rao *et. al.,* 1998) compared to all other concentrations of BAP alone and also in combination with (0.5mg/L) IAA.

Direct shoot regeneration occurred on MS medium containing BAP/Kn and in combination with IAA in hypocotyl, stem and leaf cultures of *Solanum viarum***.** They have also observed the maximum shoot regeneration on IAA+BAP, 2-iP and superiority of BAP over Kn similar to the present observation Jas Rai *et.al.,*(1999) reported the induction of large number of shoot buds from leaf disc explants of *Passiflora caerulea* when cultured on MS medium fortified with BAP+IAA Desai and Mehta (1990) have also reported direct shoot regeneration from seedling explants of *Passiflora edulis* on MS medium supplemented with BAP+ Coconut water. Similarly, maximum numbers of shoots were obtained when leaf explants of moth bean and pigeon pea were cultured on MS medium containing BAP+IAA (Kunjumon *et.al.,* 1996).

Neeta *et.al.,*(2001) have reported the direct plantlet regeneration from different explants, i.e. hypocotyl, epicotyls, cotyledon and leaves cultured on IAA+BAP combination, but they found the highest average number of shoot buds per leaf explant in *Azadhiracta indica*. Similarly it was also reported in *Capsicum annuum* (Christopher and Rajam 1996) and 7 Genotypes of *Capsicum annuum* (Venkataiah and Subhash, 2001).

As with our result, *S.torvum* large numbers of adventitious shoot buds were induced by TDZ, but not by other cytokinins (Blakesey 1990). Variation in the activity of different cytokinins can be explained by their differential uptake rate reported in different genomes.

TDZ, when used in combination with auxins, drastically reduced the percentage of response. Moreover there was no significant increase in the number of shoots regeneration per explants. In contrast, Yildirim and Turker (Blakesey1990) observed a significant increase in the percentage of explants forming shoot and the mean shoot number per explants when TDZ was used in combination with IAA.

A similar pattern of shoot bud development from leaf explants was reported by (Banerjee *et. al.,* 2004). Although it was difficult to count the number of shoot buds, it was clearly visible that the number of shoot buds increases with every increase in TDZ concentration. In lower concentrations of TDZ the shoots elongated with some expanded leaves, whereas in the higher concentration the shoot buds were highly vitrified and fail to elongate.

The efficacy of TDZ for induction of direct shoot organogenesis is well documented in several woody plants (Graham and Millam 1997, Leblay *et.al.,* 1991, Preece and Imel 1991)) Moreover some researchers (Hsia and Korban 1997) have reported TDZ to be an essential growth regulator for shoot induction from leaf explant of Ericaceae family. It was emphasized that the efficiency of TDZ may be due to its ability to induce cytokinin accumulation (Victor *et.al.,* 1999) or enhance the accumulation and translocation of auxin within the tissue (Murthy *et.al.,*1998) TDZ is also suspected of

promoting regulated morphogenesis in plants through the modulation of endogenous cytokinin and auxin (Gill and Saxena1992). Heutteman and Preece (1993) emphasized the potential use of TDZ in the regulation of adventitious shoot production and hypothesize on the synergism existing between TDZ and both endogenous and exogenous auxin.

From the foregoing discussion, it is evident that cotyledon and Leaf explants were found to be more potential in producing high frequency number of shoots among all other explants tested in the present investigation Cytokinins BAP / Kn/TDZ alone or in combination with IAA was effective in inducing shoot regeneration in all the explants of *S.torvum.* However, (3.0 mg/L) Kn / BAP with (0.5 mg/L) IAA combination induced highest number of shoots in Cotyledon and leaf explants. Thus, the plants regenerated *in vitro* by direct organogenesis may exhibit greater genetic stability than those produced from callus (Lee and Phillips, 1988).The regeneration protocols developed from Cotyledon and leaf explants in the present investigation can be used for mass propagation of the species and also for genetic manipulation studies to introduce agronomically important traits.

CONCLUSIONS

Research work has mainly been focused on the development of regeneration protocol, somaclonal variations and their physiological as well as morphological aspects in *S.torvum* Plant. An efficient plant regeneration protocol is a pre-requisite for the exploitation of various biotechnological techniques. However, practical utility of the basic protocol is still far away. It can serve as a platform for the transfer of economically important traits through genetic engineering, inducing somaclonal variations, *in vitro* mutations, double-haploids induction, development and utilization of somatic hybrids, determining herbicide or pesticide tolerance limits in *S. torvum* Plant. Therefore, a remark-able progress can be made in *S. torvum* improvement through the combination of conventional and biotechnological approaches.

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