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Meta-Topolin Induced Shoot Organogenesis and Plant Regeneration from Different Explants of Tomato (*Solanum lycopersicum* L.)

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A B S T R A C T

Tomato (*Solanum lycopersicum* L.) is the most prominent vegetable crop in the Solanaceae family. The varying concentrations of meta-Topolin (mT) evaluated at 2.0 mg/l generated the highest number of shoots than other purine-type cytokinins, like 6-Benzylaminopurine (BAP) and Kinetin (KIN) from diverse explants of tomato. The addition of Indole 3-acetic acid (IAA) at 0.1 mg/l with 2.0 mg/l mT triggered the production of the optimum number of shoots (18.6) from cotyledon than hypocotyl (13.8) and leaf (11.9) explants obtained from two genotypes of tomato cultivars. The ArkaSamrat (AS) cultivar was observed to be better responsive for induction and maximum production of shoots than the ArkaRakshak (AR) cultivar of tomato. The shoots were separated from the clusters of shoots and then cultured to the rooting medium containing IAA at 1.0 mg/l, which was found to be the most suitable concentration for induction rooting in both tomato cultivars. The complete plants were shifted to the greenhouse and recorded 93% and 89% survival rates in AS and AR cultivars, respectively. The regenerated plants did not show any variation in morphology with their mother plants. This plant regeneration system can be feasible for genetic improvement through the *Agrobacterium*-mediated transformation and generation of genome-targeted mutants using genome editing tools.

Introduction

Tomato (*Solanum lycopersicum* L.) is the second most prominent vegetable crop after potato and belongs to the *Solanaceae* family. It is extensively cultivated all over the world under diverse climatic conditions like tropical, subtropical, and temperate regions (Billah *et al.,* 2019; Stavridou *et al.,* 2019; Sandhya *et al.,* 2022; Ahmed *et al.,* 2023). The worldwide production of tomatoes was recorded as 189.14 million metric tons in 2021 (FAO, 2023). China is the leading producer, with 67.54 million metric tons total production, followed by India with 21.18 million metric tons, and other countries like Turkiye, the United States of America (USA), Italy, Egypt, Spain, Mexico, Brazil, and Nigeria are the prominent producers of tomato (FAO, 2023). Several abiotic and biotic stresses have severely reduced the global production of tomato (Sandhya *et al.,* 2022; Yesmin *et al.,* 2022; Ahmed *et al.,* 2023). The application of modern plant biotechnology methods will

be helpful in producing novel cultivars with desirable traits within a short period (Sandhya *et al.,* 2022; Ahmed *et al.,* 2023; Vats *et al.,* 2023).

The novel cultivars are resilient to different agro-climatic conditions, and various biotic and abiotic stresses will be generated by applying genetic transformation and genome editing techniques in several plant species, including tomato (Sandhya *et al.,* 2022; Chinnusamy *et al.,* 2023). A proficient plant regeneration method will help in developing genetically improved cultivars using plant molecular biology and genome editing tools in tomato (Yesmin *et al.,* 2022; Sandhya *et al.,* 2022; Vats *et al.,* 2023). There are several plant regeneration protocols have been established using several cultivars of tomato from various explant types, such as cotyledon (Alatar *et al.,* 2017; Sandhya *et al.,* 2022; Vats *et al.,* 2023; Velda *et al.,* 2023), hypocotyl (Billah *et al.,* 2019; Ahmed *et al.,* 2023; Vats *et al.,* 2023), leaf (Vinoth *et al.,* 2019; Titeli *et al.,* 2021; Sandhya *et al.,* 2022), and rhizoid tubers (Saeed *et al.,* 2019). The regeneration efficiencies depend upon several factors, such as the application and treatment of various plant growth regulators (PGRs), age and type of explant, and genotypes/cultivars (germplasm) in tomato (Billah *et al.,* 2019; Stavridou *et al.,* 2019; Sandhya *et al.,* 2022; Yesmin *et al.,* 2022; Velda *et al.,* 2023). The plant regeneration system significantly influences the genetic transformation efficiency, which includes different factors like the initiation and proliferation of shoot buds and complete plantlets recovery after rooting (Sandhya *et al.,* 2022; Yesmin *et al.,* 2022; Vats *et al.,* 2023). Therefore, proficient plant regeneration methods are required to attain maximum transformation efficiency in plant species. The manipulation of PGRs plays a critical role in the successful recovery of complete plants by inducing cell division, cell proliferation, and differentiation of meristematic cells into shoots and roots (Kieber and Schaller, 2018; Gupta *et al.,* 2020; Hurny *et al.,* 2020). Several plant regeneration protocols have been established from diverse explants of many tomato cultivars/genotypes by employing different cytokinins and auxins, either alone or in combination (Stavridou *et al.,* 2019; Sandhya *et al.,* 2022; Vats *et al.,* 2023; Velda *et al.,* 2023). Cytokinins are critical in regulating several physiological, morphological, and developmental processes, including cell division, meristem differentiation, and organ formation (Kieber and Schaller, 2018; Hurny *et al.,* 2020). The mT is a purine-type cytokinin successfully employed for initiation and proliferation of adventitious shoots and developing plant regeneration systems using different explants of several plants like *Allamanda cathartica* (Khanam *et al.,* 2020), *Salvia viridis* (Grzegorczyk-Karolak *et al.,* 2020), *Oxystelma esculentum* (Jayaprakash *et al.,* 2021), *Vanilla planifolia* (Manokari *et al.,* 2021) including the *Solanaceous* species such as *Physalis minima* (Haldar and Ghosh, 2021), *Solanum tuberosum* (Char *et al.,* 2023) and *Withania somnifera* (Kaur *et al.,* 2021).

Hence, the present study was performed to evaluate the effect of various concentrations of three purine-type cytokinins (BAP/KIN/mT) alone and in combination with auxins (IAA/IBA/NAA) on initiation and proliferation of adventitious shoots from different explants of two cultivars of tomato such as ArkaRakshak (AR) and ArkaSamrat (AS). The influence of varying concentrations of auxins (IAA/IBA/NAA) has been determined for rooting efficiency and generation of complete plantlets.

Materials and Methods

Plant material and explant preparation

Seeds of tomato (*Solanum lycopersicum* L.) cultivars, ArkaRakshak (AR) and ArkaSamrat (AS) were procured from the ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru, India. The seeds were washed under top water by adding 2-3 drops of Tween-20, then dipped in 70% ethanol for 1-2 min, followed by sodium hypochlorite (4%) for 10 min and then thoroughly rinsed in sterile water 5-6 times to eliminate the traces of sterilants and germinated on a half-strength MS (Murashige and Skoog, 1962) basal medium with 2% sucrose and solidified with 0.8% agar. The pH was adjusted to 5.8 with 0.1N NaOH or 0.1N HCl in the medium. The culture media were autoclaved for 20 min at 121^{o}C and 15 lb pressure. The cotyledon and hypocotyl explants obtained from 2-week-old and leaf explants were prepared using 4-week-old *in vitro* raised seedlings.

Culture media and conditions

All the regeneration media were prepared using MS basal salts containing 3% sucrose, varying concentrations of three purine-type cytokinins, BAP, KIN, and mT, alone and combined with auxins such as IAA/IBA/NAA(Tables 1-3) for the evaluation of shoot initiation and proliferation efficiency from different explants. The varying concentrations of auxins like

IAA/IBA/NAA (0.25. 0.5, and 1.0 mg/l) were employed to evaluate rooting efficiency. All cultures were maintained at 25 ± 2°C and with a photoperiod of 16/8 h light/dark conditions using fluorescent lights with a light intensity of 50 $\mu mol^{-2}s^{-1}$.

Shoot induction, elongation, and rooting

To evaluate the effect of different concentrations and combinations of cytokinins and auxins on the induction and elongation of shoots obtained from three explants of two tomato cultivars (ArkaRakshak-AR and ArkaSamrat-AS) (Tables 1-3). The explants were exhibited with the initiation of adventitious buds within two weeks of culture. After two to three subcultures of explants with several shoot buds, they were further increased in the media supplemented with the same concentration of PGRs after 2 to 3 rounds of subcultures on medium containing varying concentrations of mT (0.5 mg/l), BAP (0.5 mg/l), Gibberellic acid (GA3) (0.1 or 0.5 mg/l) and IAA (0.1 or 0.5 mg/l) alone or in combinations for elongation of shoots from clumps of multiple shoot buds. The elongated shoots (more than 2 cm long) were separated from clumps of shoots and transferred to the rooting medium augmented with auxins like IAA, IBA, and NAA (0.25 to 1.0 mg/l). The medium without auxins is considered a control for evaluating different auxin concentrations of rooting.

Acclimatization of regenerated plants

The well-developed plantlets were taken out from culture bottles and then washed under the tap water to take away the culture medium sticking with roots and then dipped in the bavistin (10% w/v) solution for 10 min and placed in paper cups containing sterilized soil-rite, sand, and soil in equal ratios. The plantlets were incubated in the culture room for acclimatization by covering them with a polythene cover containing small holes for two weeks. The acclimatized plants were placed in the greenhouse for further establishment and maturation. After six weeks of acclimatization, the data on the survival of plants were scored and analyzed.

Data analysis

All the experiments were performed thrice, eachwith30 explants per treatment. The data on the number of shoot buds induced from each explant and each treatment was recorded using the stereomicroscope. All the data generated from each treatment and each explant were analyzed using mean ± standard error, presented in tabular form, and the significant levels were compared using Duncan's multiple range test (DMRT) at $P = 0.05$ using SPSS 20 software (SPSS Inc, USA).

Results and Discussion

Several new genotypes/cultivars of tomato have been developed using conventional breeding methods. Classical breeding techniques require intensive labor, are time-consuming, and take many generations to produce novel germplasm (Tiwari *et al.,* 2023). Hence, the molecular breeding methods have been considered the most suitable for the improvement of the germplasm of tomato (Van Eck, 2020). The successful application of these techniques depends on the proficient and reproducible regeneration system in numerous cultivars of tomato (Alatar *et al.,* 2017; Sandhya *et al.,* 2022; Yesmin *et al.,* 2022; Vats *et al.,* 2023; Velda *et al.,* 2023; Yaroshko *et al.,* 2023). Therefore, the present study has been carried out to establish a reproducible plant regeneration protocol in two cultivars of tomato (AR and AS) using cotyledon, hypocotyl, and leaf explants. The present study also examines the effect of different factors on plant regeneration efficiency, which depends on several factors like explant type, genotypes/cultivars, and plant growth regulators (PGRs), and their concentrations and combinations of tomato (Titeli *et al.,* 2021; Sandhya *et al.,* 2022; Yesmin *et al.,* 2022; Velda *et al.,* 2023; Yaroshko *et al.,* 2023). The role of different concentrations of PGRs on the induction and proliferation of shoots and subsequent generation of complete plants from different explants was evaluated in several cultivars of tomato (Titeli *et al.,* 2021; Sandhya *et al.,* 2022; Yesmin *et al.,* 2022; Vats *et al.,* 2023; Velda *et al.,* 2023; Yaroshko *et al.,* 2023).

Effect of cytokinins on shoot induction

The influence of different factors like explants, genotypes, concentrations, and combinations of PGRs on plant regeneration efficiency was determined by using three types of explants of two tomato genotypes (AR and AS) and three different purine-type cytokinins (BAP, KIN, and mT) individually. The three types of explants were inoculated on a medium without hormone supplementation and did not show any response except little callus formation at cut ends. The explants showed the induction of shoot buds when cultured on media added with different cytokinin concentrations (Tables 1-3). The cytokinins are supposed to be involved in many

physiological and developmental functions, like cell division, proliferation, and differentiation into the shoot and/or root primordia (Kieber and Schaller, 2018). The shoot bud initiation from all explants was observed within 8-14 days of culture, depending upon the type of explant, cytokinin type, and genotype/cultivar. The explants exhibited the initiation of shoot buds after a week on a medium augmented with either BAP or mT. In contrast, the KIN-fortified medium showed a delayed response in initiating shoot buds from explants. Among the two genotypes assessed, the AS genotype was found to be more responsive than the AR genotype. Our observations agree with earlier reports on tomato, where different genotypes, explants, and cytokinins showed diverse regeneration responses (Titeli *et al.,* 2021; Sandhya *et al.,* 2022; Yesmin *et al.,* 2022; Velda *et al.,* 2023).

The shoot induction medium fortified with various concentrations of mT (0.5 to 2.5 mg/l) induced a varied number of shoots depending on the concentrations of mT. The medium containing mT at 2.0 mg/l yielded an optimal number of shoots and maximum regeneration efficiency for all the explants in both tomato cultivars (Tables 1-3). The shoot induction response and the number of shoots increased gradually. The concentration of up to 2.0 mg/l of mT and then reduced the number of shoots and regeneration percentage with increased concentration of mT in both genotypes (Tables 1-3). Among the cytokinins employed, the mT was recorded as the best responsive cytokinin compared to other cytokinins like BAP and KIN in all three explants of two cultivars. The regeneration containing mT at 2.0 mg/l was observed to be an efficient concentration for initiating the optimum number of shoots and regeneration frequency in all explants types (Tables 1-3). The regeneration medium containing mT at 2.0 mg/l was more efficient cytokinin than BAP and KIN, producing from a range of 1.6 to 8.6 shoots relied on the explants in AR and AS genotypes after six weeks of cultures (Tables 1-3). The cotyledon explants produced 8.6 and 6.8 shoots on regeneration medium fortified with mT (2.0 mg/l) after six weeks in AS and AR, respectively. The hypocotyl explants generated 6.8 and 5.9 shoots. In contrast, leaf explants induced an average of 5.8 and 4.7 shoots on medium added with mT (2.0 mg/l) in the AS and AR cultivars, respectively. The medium includes BAP at 2.0 mg/l, which recorded 7.9 shoots/cotyledon, 6.6 shoots/hypocotyl, and 6.3 shoots/leaf explants of the AS genotype. In contrast, the AR genotype generated 6.3 shoots/cotyledon, 5.8 shoots/hypocotyl, and 4.8 shoots/leaf (Tables 1-3). The medium supplemented with KIN at 2.0 mg/l was the optimal concentration for induction of shoots in both cultivars, recorded as cotyledon explants produced 5.4 and 3.8 shoots in the AS and AR genotypes, respectively. In comparison, 4.9 and 3.5 shoots in hypocotyl and 4.6 and 3.6 shoots in leaf were observed in AS and AR genotypes, respectively (Tables 1-3).

The application of individual purine-type cytokinins (BAP, KIN, and mT) differed in inducing and proliferation of adventitious shoots and plant regeneration efficiency in various plants of the *Solanaceae* family (Haldar and Ghosh, 2021; Kaur *et al.,* 2021; Char *et al.,* 2023) and diverse explant types of tomato cultivars (Al-Kaaby, 2016; Billah *et al.,* 2019; Sandhya *et al.,* 2022; Yesmin *et al.,* 2022; Ahmed *et al.,* 2023). The regeneration medium containing varying BAP/KIN concentrations initiated multiple shoots and plant regeneration in diverse explants of tomato (Billah *et al.,* 2019; Yesmin *et al.,* 2022; Sandhya *et al.,* 2022). Our observations are in concurrence with earlier recorded results on the efficiency of various cytokinins used in the development of plant regeneration systems from a wide variety of tomato cultivars (Billah *et al.,* 2019; Sandhya *et al.,* 2022; Velda *et al.,* 2023). Among varying concentrations of mT, 2.0 mg/l of mT was shown to be the most appropriate for induction of the maximum number of multiple shoots in both tomato cultivars. The mT (2.0 mg/l) was observed as more efficient than other cytokinins (BAP/KIN) for the production of multiple shoots and developing repeatable plant regeneration systems in *Allamanda cathartica* (Khanam *et al.,* 2020), *Salvia viridis* (Grzegorczyk-Karolak *et al.,* 2020), *Physalis minima* (Haldar and Ghosh, 2021), *Oxystelma esculentum* (Jayaprakash *et al.,* 2021), *Vanilla planifolia* (Manokari *et al.,* 2021), *Withania somnifera* (Kaur *et al.,* 2021) and *S. tuberosum* (Char *et al.,* 2023).

Effect of auxin and cytokinin on shoot induction

The cytokinin and auxin interaction involves several developmental stages, such as the initiation, multiplication, and proliferation of shoots and complete plant regeneration in many plants (Gupta *et al.,* 2020; Long *et al.,* 2022). Plant regeneration significantly depends on several factors, such as explant, genotype, PGRs, and the addition of other substances that affect the initiation and proliferation of shoots (Long *et al.,* 2022). The addition of diversified concentrations of IAA, IBA, and NAA (0.1 to 1.00 mg/l) in combination with

cytokinins (BAP, KIN, and mT) was employed to determine their role in the formation and growth of shoots from various explants of both cultivars. The supplementation of two auxins, like IBA and NAA, does not improve the induction of shoots in all the explants of two tomato genotypes (Data not shown). Meanwhile, adding different concentrations of IAA (0.1 to 1.0 mg/l) with all cytokinins individually significantly enhanced the induction of multiple shoot buds in both tomato cultivars. Among the different concentrations of IAA evaluated, IAA at 0.1 mg/l was an efficient combination for the maximum shoots obtained from different explants of two varieties of tomato (Tables1-3).

The AR genotype explants showed various responses in the induction of multiple shoots on the medium containing various cytokinins and diverse concentrations of IAA. The medium with BAP at 2.0 mg/l and IAA at 0.1 mg/l combination produced the maximum number of shoots as 13.8 shoots/cotyledon, 10.8 shoots/hypocotyl, and 10.2 shoots/leaf explants. Meanwhile, the AS genotype showed a significantly increased number of shoots at 15.6 shoots/cotyledon, 12.6 shoots/hypocotyl, and 10.8 shoots per leaf explant. The combination of KIN at 2.0 mg/l and IAA at 0.1 mg/l recorded the maximum number as 9.3 shoots/cotyledon, 8.7 shoots/hypocotyl, and 7.6 shoots/leaf explant in the AS genotype (Tables1-3). In contrast, there was a considerably enhanced number of shoots at 8.6 shoots/cotyledon, 7.8 shoots/hypocotyl, and 6.9 shoots/leaf explants of AR genotype (Tables1-3). The two cultivars and three explants showed different regeneration responses, such as the initiation of adventitious shoots on the medium augmented with mT and varying concentrations of IAA. The medium fortified with mT (2.0 mg/l) and IAA (0.1 mg/l) exhibited a significantly increased number of shoots as the AS genotype showed an enhanced number of shoots, like 18.6 shoots/cotyledon (Fig. 1a), 13.8 shoots/hypocotyls (Fig 1b), and 11.9 shoots/leaf (Fig. 1c). Meanwhile, 15.7 shoots/cotyledon, 11.4 shoots/hypocotyl, and 10.9 shoots/leaf explants of the AR genotype (Table 1-3).

The varying cytokinin concentrations and different auxins significantly increased numerous adventitious shoots and the proliferation of shoots from explants and several tomato cultivars. The BAP in combination with IAA (Arulananthu *et al.,* 2019; Saeed *et al.,* 2019; Yesmin *et al.,* 2022; Sandhya *et al.,* 2022; Ahmed *et al.,* 2023), IBA (Vinoth *et al.,* 2019; Hashmi *et al.,* 2022), and NAA (Saeed *et al.,* 2019; Vinoth *et al.,* 2019) induced multiple shoots from different explants of several varieties of tomato. The medium fortified with diverse concentrations of KIN and IAA/NAA initiated numerous shoots and plant regeneration in a wide array of explants of tomato (Billah *et al.,* 2019; Vinoth *et al.,* 2019; Saeed *et al.,* 2019; Yesmin *et al.,* 2022). Combining IAA with mT effectively induced multiple shoots and increased their number from all three explants of both cultivars. Our findings are in concurrence with earlier results on the efficiency of mT on the multiple shoot induction in several plants like *Allamanda cathartica* (Khanam *et al.,* 2020), *Salvia viridis* (Grzegorczyk-Karolak *et al.,* 2020), *Oxystelma esculentum* (Jayaprakash *et al.,* 2021), *Vanilla planifolia* (Manokari *et al.,* 2021) and as well as the *Solanaceous* species like *Physalis minima* (Halder and Ghosh, 2021), *Withania somnifera* (Kaur *et al.,* 2021) and *Solanum tuberosum* (Char *et al.,* 2023). The regeneration containing mT induced a significant number of healthy shoots with the highest regeneration efficiency from three explant types of two varieties of tomato.

Shoot elongation and rooting

The elongation of adventitious shoots obtained from different explants is a significant bottleneck and affects the recovery of plantlets. The explants produced several tiny shoot buds (<2 mm length counted using the stereomicroscope) on various concentrations and combinations of PGRs (Tables 1-3). The many adventitious buds produced from explants were not elongated on the shoot induction medium. The explants containing the clusters of tiny shoots were transferred to different concentrations and combinations of media. The explants with tiny shoot buds were cut into pieces with ~5-10 shoot buds and then moved to media amended with alone or varying combinations of mT(0.5 mg/l), BAP (0.5 mg/l), GA3 (0.1 or 0.5 mg/l), and IAA (0.1 or 0.5 mg/l). The varying concentrations and combinations have significantly enhanced the shoot elongation in all explants of two tomato varieties.

The combination of elongation medium containing mT (0.5 mg/l), IAA (0.5 mg/l), and GA3 (0.5 mg/l) recorded the highly efficient combination for shoot elongation in tomato (Fig. 1 d). The cotyledon explants showed a 94.6% response, the hypocotyl explant exhibited an 89.4% response, and leaf explants recorded an 86.2% response, with an average shoot length of 6.4 cm in the AS genotype. The cotyledons showed a 91.8% response in the AR genotype.

Table.1 Influence of different plant growth regulators (PGRs) on shoot induction from cotyledon explants of two cultivars of tomato (*Solanum lycopersicum* L.)*

Plant growth regulators (mg/L)	ArkaRakshak (AR)		ArkaSamrat (AS)	
	% explants responding (Mean ± SE)	No. of shoots/ explant (Mean ± SE)	% explants responding (Mean ± SE)	No. of shoots/ Explant (Mean ± SE)
BAP				
0.5	47.8±1.54^{e}	1.8±0.49^{de}	52.6±1.66^{e}	2.6±0.76^{de}
1.0	63.2±1.76^{d}	2.4±0.64^{cd}	68.7±1.82^{d}	3.4±0.78^{cd}
1.5	69.7±1.72^{c}	3.6±0.77^{bc}	73.4±1.64^{c}	5.8±1.24^{bc}
2.0	86.4±1.48^{ab}	6.3±0.92^{ab}	89.5±1.86^{ab}	7.9±1.65^{ab}
2.5	83.3±1.66^{ab}	5.8±0.83^{ab}	85.7±1.75^{ab}	7.2±1.48^{ab}
KIN				
0.5	38.2±1.73^{e}	1.4±0.67^{de}	43.8±1.84^{e}	1.6±0.82^{de}
1.0	44.7±1.82^{d}	2.1±0.72^{cd}	47.4±1.76^{d}	2.7±0.68^{cd}
1.5	65.3±1.76^{c}	2.8±1.36^{bc}	70.6±1.68^{c}	3.6±1.23^{bc}
2.0	80.6±1.45^{ab}	3.8±1.57^{ab}	86.6±1.56^{ab}	5.4±1.49^{ab}
2.5	78.5±1.53^{ab}	3.5±1.75^{ab}	83.8±1.78^{ab}	5.2±1.64^{ab}
mT				
0.5	52.3±1.61^{e}	1.6±1.03^{de}	54.8±1.77^{e}	2.5±1.39^{de}
1.0	66.8±1.75^{d}	3.4±1.42^{cd}	67.3±1.83^{d}	4.6±1.48^{cd}
1.5	78.6±1.76^{c}	4.7±1.58^{bc}	82.7±1.58^{bc}	6.4±1.59^{bc}
2.0	87.5±1.63^{ab}	6.8±1.73^{ab}	89.6±1.91^{ab}	8.6±1.78^{ab}
2.5	84.2±1.72^{ab}	6.4±1.34^{ab}	86.9±1.87^{ab}	7.5±1.65^{ab}
BAP + IAA				
2.0 + 0.1	94.6±1.65^{ab}	13.8±1.34^{ab}	96.2±1.56^{ab}	15.6±1.67^{ab}
2.0 + 0.2	91.5±1.78^{bc}	11.9±1.46^{ab}	93.8±1.43^{bc}	12.7±1.86^{bc}
2.0 + 0.5	89.6±1.76^{bc}	10.6±1.72^{bc}	92.5±1.58^{bc}	12.1±1.47^{bcd}
2.0 + 1.0	87.4±1.66^{cd}	9.7±1.83^{cd}	91.6±1.66^{cd}	10.8±1.58^{cd}
KIN + IAA				
2.0 + 0.1	90.7±1.71^{ab}	8.6±1.43^{ab}	91.8±1.79^{ab}	9.3±1.64^{ab}
2.0 + 0.2	89.2±1.86^{ab}	7.5±1.56^{ab}	90.9±1.68^{bc}	8.4±1.62^{bc}
2.0 + 0.5	88.4±1.67^{bc}	5.8±1.63^{bc}	89.7±1.64^{cd}	6.5±1.68^{cd}
2.0 + 1.0	85.6±1.82^{cd}	5.4±1.72^{cd}	86.9±1.73^{de}	4.8±1.72^{de}
mT + IAA				
2.0 + 0.1	95.8±1.72^{ab}	15.7±1.82^{ab}	97.6±1.84^{f}	18.6±1.72^{ab}
2.0 + 0.2	93.4±1.68^{ab}	12.9±1.68^{ab}	96.4±1.56^{e}	15.5±1.68^{ab}
2.0 + 0.5	91.6±1.73^{bc}	10.7±1.73^{bc}	95.7±1.67^{d}	12.4±1.57^{bc}
2.0 + 1.0	91.2±1.96^{bc}	10.3±1.56^{cd}	92.8±1.78^{bc}	11.6±1.71^{cd}

*Each experiment was performed three times with 30 replicates. The data was analyzed and presented in tabular form in mean ± standard error. The mean values with the same letter within columns are not significantly different according to Duncan's New Multiple Range Test at a 5% level (P>0.05).

Table.2 Influence of different plant growth regulators (PGRs) on shoot induction from hypocotyl explants of two cultivars of tomato (*Solanum lycopersicum* L.)*

Plant growth regulators (mg/L)	ArkaRakshak (AR)		ArkaSamrat (AS)	
	% explants responding (Mean ± SE)	No. of shoots/ explant (Mean ± SE)	% explants responding (Mean ± SE)	No. of shoots/ explant (Mean ± SE)
BAP				
0.5	38.7±1.48^{e}	1.2±0.73^{de}	45.2±1.56^{e}	1.5±1.47^{de}
1.0	52.6±1.65^{d}	2.3±1.24^{cd}	56.3±1.93^{d}	2.6±1.69^{cd}
1.5	62.9±1.87^{c}	3.4±1.62^{bc}	68.5±1.64^{c}	3.9±1.82^{bc}
2.0	74.6±1.84^{a}	5.8±1.67^{ab}	79.7±1.76^{ab}	6.6±1.79^{ab}
2.5	66.8±1.56^{b}	4.7±1.73^{ab}	77.8±1.82^{ab}	5.4±1.86^{ab}
KIN				
0.5	34.8±1.83^{e}	1.1±1.03^{de}	39.4±1.76^{e}	1.3±1.78^{cd}
1.0	43.4±1.68^{d}	2.2±1.37^{cd}	52.8±1.82^{d}	2.7±1.65^{bc}
1.5	61.8±1.57^{c}	2.8±1.68^{bc}	67.6±1.91^{c}	3.4±1.62^{bc}
2.0	73.6±1.84^{ab}	3.8±1.81^{ab}	76.9±1.67^{ab}	4.9±1.87^{ab}
2.5	68.7±1.92^{ab}	3.2±1.76^{ab}	72.3±1.58^{ab}	4.2±1.76^{ab}
mT				
0.5	58.6±1.82^{e}	1.6±1.35^{de}	66.5±1.62^{e}	2.2±1.34^{de}
1.0	67.5±1.84^{d}	3.3±1.56^{bcd}	72.3±1.63^{d}	3.5±1.67^{cd}
1.5	77.3±1.76^{bc}	3.8±1.74^{bc}	79.8±1.85^{bc}	4.9±1.78^{bc}
2.0	81.8±1.74^{ab}	5.9±1.86^{ab}	84.4±1.78^{ab}	6.8±1.64^{ab}
2.5	80.6±1.85^{ab}	5.2±1.72^{ab}	81.6±1.82^{ab}	5.6±1.56^{ab}
BAP + IAA				
2.0 + 0.1	91.6±1.74^{ab}	10.8±1.68^{ab}	92.8±1.67^{ab}	12.6±1.32^{ab}
2.0 + 0.2	90.7±1.83^{ab}	09.4±1.73^{abc}	91.3±1.63^{ab}	10.2±1.67^{ab}
2.0 + 0.5	86.7±1.76^{cd}	08.6±1.58^{bcd}	89.6±1.76^{bc}	09.5±1.92^{bc}
2.0 + 1.0	85.4±1.66^{cd}	07.5±1.82^{bcd}	88.9±1.82^{bcd}	08.4±1.68^{bcd}
KIN + IAA				
2.0 + 0.1	89.4±1.67^{a}	07.8±1.74^{ab}	90.5±1.91^{ab}	08.7±1.67^{ab}
2.0 + 0.2	85.6±1.72^{bc}	06.7±1.65^{abc}	87.8±1.84^{ab}	07.3±1.56^{ab}
2.0 + 0.5	84.3±1.62^{bc}	06.2±1.72^{bc}	86.4±1.76^{bc}	06.9±1.73^{bc}
2.0 + 1.0	80.1±1.68^{d}	05.6±1.46^{cd}	84.5±1.68^{cd}	06.1±1.68^{bc}
mT + IAA				
2.0 + 0.1	93.4±1.82^{ab}	11.4±1.75^{ab}	94.6±1.64^{ab}	13.8±1.68^{ab}
2.0 + 0.2	92.6±1.76^{abc}	10.8±1.82^{ab}	93.5±1.78^{ab}	11.4±1.57^{ab}
2.0 + 0.5	91.8±1.69^{bc}	09.4±1.68^{bc}	91.2±1.83^{bc}	10.7±1.74^{bc}
2.0 + 1.0	89.6±1.74^{cd}	08.7±1.59^{cd}	90.4±1.92^{cd}	10.2±1.83^{bc}

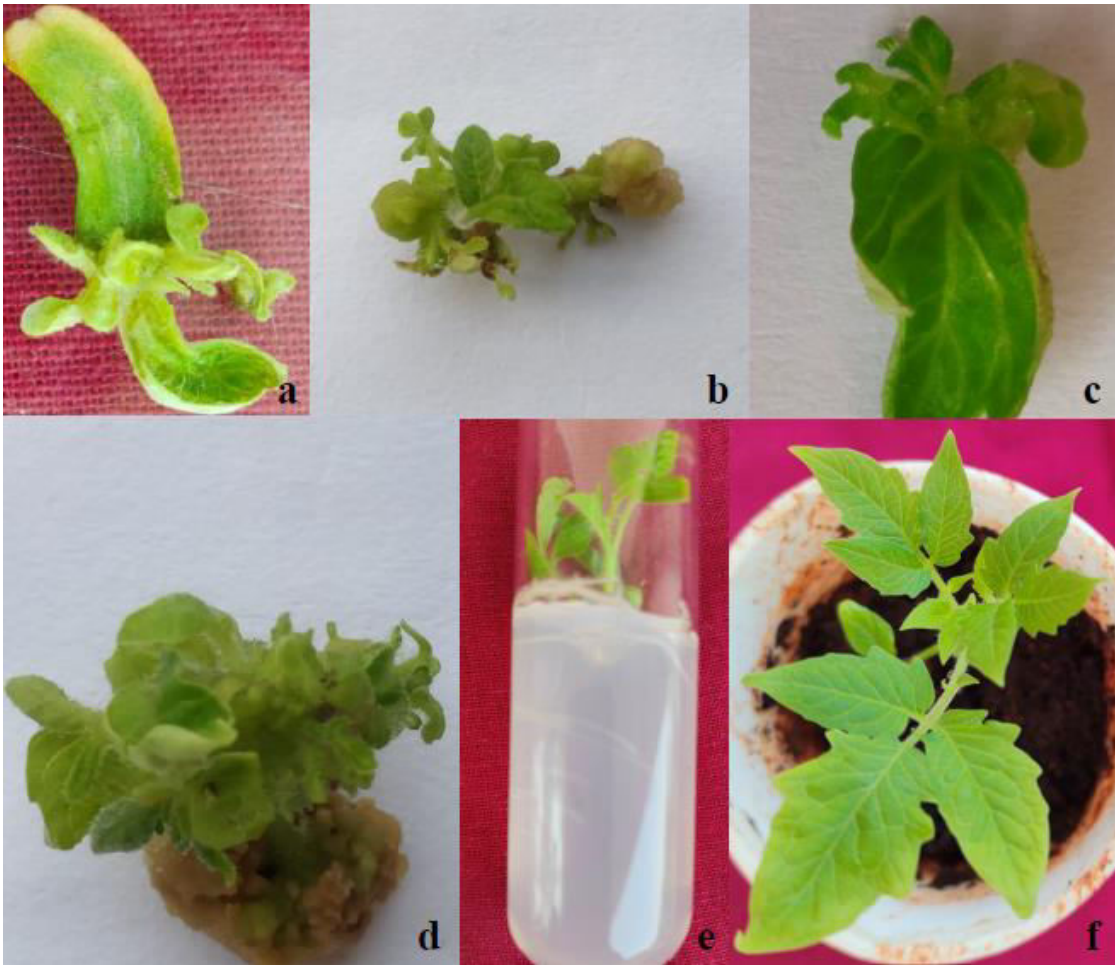
*Each experiment was performed three times with 30 replicates. The data was analyzed and presented in tabular form in mean ± standard error. The mean values with the same letter within columns are not significantly different according to Duncan's New Multiple Range Test at a 5% level (P>0.05).

Table.3 Influence of different plant growth regulators (PGRs) on shoot induction from leaf explants of two cultivars of tomato (*Solanum lycopersicum* L.)*

Plant growth regulators (mg/L)	ArkaRakshak (AR)		ArkaSamrat (AS)	
	% explants responding (Mean ± SE)	No. of shoots/ explant (Mean ± SE)	% explants responding (Mean ± SE)	No. of shoots/ explant (Mean ± SE)
BAP				
0.5	34.8±1.57^{e}	1.6±1.14^{de}	42.5±1.66^{e}	2.4±1.32^{de}
1.0	49.7±1.92^{d}	2.5±1.57^{bcd}	55.8±1.79^{d}	3.2±1.59^{cd}
1.5	76.6±1.65^{c}	3.3±1.65^{bc}	79.6±1.82^{bc}	4.4±1.63^{bc}
2.0	81.8±1.77^{ab}	4.8±1.83^{ab}	82.2±1.71^{ab}	6.3±1.76^{ab}
2.5	78.7±1.93^{ab}	4.4±1.59^{ab}	80.4±1.68^{ab}	5.2±1.68^{ab}
KIN				
0.5	32.4±1.92^{e}	1.2±1.23^{bcd}	36.9±1.64^{e}	2.1±1.44^{cde}
1.0	41.6±1.86^{d}	2.3±1.76^{bc}	48.5±1.78^{d}	2.8±1.68^{cd}
1.5	59.1±1.72^{c}	3.2±1.68^{ab}	64.7±1.66^{c}	4.2±1.73^{bc}
2.0	71.8±1.81^{a}	3.8±1.82^{ab}	74.8±1.76^{ab}	5.4±1.76^{ab}
2.5	64.3±1.69^{b}	3.3±1.76^{ab}	71.2±1.89^{ab}	4.3±1.82^{ab}
mT				
0.5	47.8±1.75^{e}	2.1±1.26^{de}	66.5±1.62^{e}	2.8±1.46^{de}
1.0	69.2±1.82^{d}	3.4±1.45^{cd}	74.6±1.71^{d}	4.2±1.65^{cd}
1.5	77.8±1.73^{bc}	4.5±1.37^{bc}	80.2±1.64^{bc}	5.4±1.71^{bc}
2.0	83.2±1.65^{ab}	5.7±1.69^{ab}	86.7±1.82^{ab}	6.6±1.84^{ab}
2.5	81.5±1.93^{ab}	5.4±1.74^{ab}	83.4±1.68^{ab}	5.8±1.69^{ab}
BAP + IAA				
2.0 + 0.1	88.2±1.76^{ab}	10.2±1.76^{ab}	91.5±1.82^{ab}	10.8±1.56^{ab}
2.0 + 0.2	86.8±1.69^{ab}	08.9±1.48^{abc}	87.8±1.73^{ab}	09.6±1.69^{ab}
2.0 + 0.5	84.2±1.73^{bc}	08.3±1.67^{bc}	86.4±1.69^{bcd}	08.6±1.82^{bc}
2.0 + 1.0	83.6±1.82^{cd}	06.8±1.58^{cd}	85.7±1.62^{bcd}	07.1±1.71^{cd}
KIN + IAA				
2.0 + 0.1	83.4±1.74^{ab}	06.9±1.46^{ab}	87.6±1.66^{ab}	07.6±1.58^{ab}
2.0 + 0.2	81.5±1.81^{ab}	06.6±1.57^{ab}	85.8±1.53^{ab}	06.9±1.49^{ab}
2.0 + 0.5	80.8±1.64^{bc}	05.8±1.73^{bc}	83.7±1.68^{bc}	06.5±1.52^{abc}
2.0 + 1.0	78.6±1.82^{cd}	05.3±1.68^{bcd}	81.6±1.69^{cd}	05.2±1.64^{cd}
mT + IAA				
2.0 + 0.1	89.6±1.68^{ab}	10.9±1.68^{ab}	91.4±1.76^{ab}	11.9±1.46^{ab}
2.0 + 0.2	87.3±1.83^{ab}	09.5±1.71^{ab}	90.5±1.68^{ab}	10.5±1.68^{ab}
2.0 + 0.5	84.5±1.72^{bc}	08.6±1.58^{bc}	87.8±1.71^{bc}	09.3±1.59^{bc}
2.0 + 1.0	83.7±1.68^{cd}	07.2±1.63^{cd}	86.3±1.56^{cd}	08.4±1.71^{bcd}

*Each experiment was performed three times with 30 replicates. The data was analyzed and presented in tabular form in mean ± standard error. The mean values with the same letter within columns are not significantly different according to Duncan's New Multiple Range Test at a 5% level (P>0.05).

Figure.1 Initiation of adventitious shoots and plant regeneration from different explants of tomato cv. ArkaSamrat (AS) on medium containing mT(2.0 mg/l) and IAA (0.5 mg/l).



Initiation of multiple shoots from (a) cotyledon, (b) hypocotyl, and (c) leaf, (d) proliferation and elongation of multiple shoots, (e) rooting of shoot on MS medium fortified with IAA (1.0 mg/l), (f) acclimatized plant under greenhouse conditions after 4 weeks.

The hypocotyl recorded an 82.3% response, and leaf explants had a 78.6% response. The elongated shoots had an average shoot length of 5.2 cm after two weeks of culture, and each subculture had a week interval.

The shoot elongation medium fortified with GA3 alone or combined with cytokinin and/or auxin plays a vital role in the elongation of healthy and normal shoots from the cluster of adventitious buds produced from different explants. The addition of different concentrations of cytokinin plays a critical role in the elongation of shoots by affecting cell division and cell expansion (Gupta and Van Eck, 2016; Gupta *et al.,* 2020; Long *et al.,* 2022). The combinations of multiple PGRs have been reported in the elongation and proliferation of shoots in several explants of a wide variety of tomato cultivars, such as on medium fortified with diverse GA3 concentrations in the addition of ZEA (Godishala *et al.,* 2012), KIN (Banu *et al.,* 2017) and BAP (Hashmi *et al.,* 2022). The shoot elongation has been observed on medium containing BAP (1.0 mg/1), IAA (0.5 mg/L), KIN (0.5 mg/1), and GA3 (0.1 mg/l) in several tomato genotypes (Banu *et al.,* 2017). The medium containing BAP (1.5 mg/1), IBA (1.5 mg/l), and GA3 (0.2 mg/l) successfully induced shoot elongation in tomato (Hashmi *et al.,* 2022). The combination of PGRs like ZEA, IAA, and GA3 has been reported for the shoot elongation and proliferation of multiple shoots produced indifferent explants of tomato (Sandhya *et al.,* 2022).

The individual shoots (>2 cm) were separated from the elongated multiple shoot bunches and moved onto the medium fortified with different auxins (IAA/IBA/NAA) (0.25 to 1.0 mg/l) for induction roots. The root initiation was observed within 10-15 days of culture, which significantly depends upon the type and concentration of auxin and genotype of tomato.

The auxin concentration of more than 1.0 mg/l did not increase the rooting efficiency and the number of roots per shoot in both genotypes. The medium fortified with IAA at 1.0 mg/l was the best auxin concentration for the optimum number of roots (Fig. 1e), which induced an average number of 16.8 roots per shoot with 97.8% rooting response in the AS genotype and an average of 12.6 roots/shoot and 86.5% of the rooting response was exhibited in the AR genotype.

The rooting frequency and the number of roots per shoot were recorded as an average of 11.4 roots per shoot and 94.6% rooting frequency in the AS genotype and 83.8% with 9.6 roots per shoot was observed in the AR cultivar on the rooting medium amended with IBA at 1.0 mg/l. The medium fortified with NAA at 1.0 mg/l produced 7.8 roots per shoot and 82.4% rooting response, and 7.2 roots per shoot with 79.8% rooting frequency was observed in the AS and AR genotypes of tomato. The IAA was more efficient than IBA and NAA among the three auxins examined.

The best rooting efficiency has been recorded in shoots obtained from the AS genotype compared to the AR genotype. The auxins play a critical role in the induction of root apical meristem initiation, growth, and other development processes in plants (Kieber and Schaller, 2018; Petrasek *et al.,* 2019; Long *et al.,* 2022). Among various evaluated, the IAA was observed as an efficient auxin for root induction and had a higher rooting frequency than IBA and NAA. The IAA was the most significant auxin type for efficient root induction in tomato genotypes (Titeli *et al.,* 2021; Vats *et al.,* 2023).

In contrast, IBA was found to be the efficient auxin for initiating the rooting in several tomato genotypes (Vinoth *et al.,* 2019; Hashmi *et al.,* 2022; Sandhya *et al.,* 2022). Similarly, the supplementation of NAA was found to be an efficient auxin in several genotypes of tomato (Vinoth *et al.,* 2019; Saeed *et al.,* 2019; Hashmi *et al.,* 2022; Sandhya *et al.,* 2022), but it is less efficient compared to other auxins such as IAA and IBA in the AS and AR genotypes of tomato.

Acclimatization of regenerated plants

The plantlets were kept in the culture room for one to two weeks for hardening or until the formation of a new leaf, then shifted to pots and moved to the greenhouse. A total of 108 plants and 124 plants produced from various explants of the AR and AS cultivars were transferred to the greenhouse for further establishment and growth of *in vitro* regenerated plants. Ninety-seven (97) out of 108 plants of the AS genotype and 116out of 124 plants obtained from the AS genotype were successfully acclimatized and established under greenhouse conditions (Fig.1f). The survival rate was 89% for the AR genotype, and a 93% survival rate for the AS genotype was recorded after six weeks of acclimatization. The plants obtained from various explants of both cultivars were phenotypically similar to their mother plants. In the present study, we have recorded the highest survival rate, which confirms the efficiency of different cytokinins like mT followed by BAP and KIN in increasing the acclimatization and survival rate of *in vitro* regenerated plants in several species like *Allamanda cathartica* (Khanam *et al.,* 2020), *Salvia viridis* (Grzegorczyk-Karolak *et al.,* 2020), *Physalis minima* (Halder and Ghosh, 2021), *Oxystelma esculentum* (Jayaprakash *et al.,* 2021), *Withania somnifera* (Kaur *et al.,* 2021), *Vanilla planifolia* (Manokari *et al.,* 2021) and *Solanum tuberosum* (Char *et al.,* 2023).

The present study demonstrates a proficient and reproducible plant regeneration system for two tomato genotypes (ArkaRakshak and ArkaSamrat) using cotyledon, hypocotyl, and leaf explants. Among various purine-type cytokinins evaluated, the mT (2.0 mg/l) was found suitable for the induction of adventitious shoots from different explants of two cultivars. Among different PGRs examined, the combination of mT (2.0 mg/l) and IAA (0.1 mg/l) was most appropriate for inducing the highest number of shoots from all explants of two tomato genotypes. IAA at 1.0 mg/l was the most suitable auxin for maximum rooting efficiency. The plant regeneration protocol developed in two tomato genotypes will be helpful for the transformation of novel candidate genes and the application of various genome editing tools for genetic improvement.

Author Contribution

Vasudha Marapaka: Investigation, formal analysis, writing—original draft. Kranthikumar Gande: Validation,

methodology, writing—reviewing. Vaishnavi Anumula:—Formal analysis, writing—review and editing. Venkataiah Peddaboina: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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