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STUDIES ON *IN-VITRO* PROPAGATION IN TURMERIC CV. PDKV WAIGAON

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ABSTRACT

The present investigation entitled "Studies on *in-vitro* propagation in turmeric cv. PDKV Waigaon" was conducted at Department of Vegetable Science, Dr. PDKV, Akola and Centre of Excellence, Plant Biotechnology, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, (M.S.) during the academic year 2021-2022 and 2022-2023 with the aim *to standardized the protocol for in-vitro propagation of turmeric* with (CRD) Completely Randomized Design having 5 treatments and 4 replications of surface sterilization of turmeric explant, *in-vitro* shoot induction and hardening of *in-vitro* grown plantlets and 3 treatments and 7 replications of *in-vitro* root induction.

In this study treatment of Bavistin 0.1% for 4 min + $HgCl_2$ 0.2% for 4 min of time showed highest survival percent of turmeric explants. When, rhizome buds were cultured on MS media with different combinations of BAP and NAA, the highest number of shoots and number of leaves per shoot was recorded by treatment MS media + 2mg/l BAP + 1 mg/l NAA, whereas, minimum number of days for shoot initiation and days for shoot proliferation after shoot initiation were also recorded by the same treatment.

In *in-vitro* root induction half strength of MS medium was used with different combinations of IBA, the highest number of roots per plant and the lowest days for root initiation were observed in treatment ½MS media + 1mg/l IBA. In the hardening of *in-vitro* grown plantlets of turmeric maximum survival percentage of plantlets, maximum plant height and number of leaves after one month of hardening was recorded in treatment of peatmoss.

In the present study above treatments were found suitable for the successful propagation of turmeric with the help of tissue culture technique.

***Key words*:** Turmeric, Plant tissue culture, Propagation, Media, *In-vitro*, etc.

Introduction

Turmeric (*Curcuma longa* L.), a herbaceous perennial herb native to Asia, belongs to the Zingiberaceae family. Among the world's most valuable spices is this one. In addition, behind chillies, it is the second most significant spice crop. India is the world's biggest producer, consumer, and exporter of turmeric. More than 75% of the world's turmeric is produced in India, which also holds a 60% export share. Minerals (3.5%), carbs (69.4%), fat (5.1%), protein (6.3%), and moisture (13.1%) are all present in turmeric. Curcumin (diferuloy|methene), which is made up of curcumin I (94%) and curcumin II (6%) and III (0.3%), is what gives it its yellow color (Ruby *et al*., 1995).

The primary difficulties in growing turmeric are that no single variety can yield more and have a curcumin concentration high enough (≥ 6%) to be exported. 90% of turmeric growing regions are devoted to long-duration cultivars, which are well-known for having a low curcumin concentration. Some varieties, including Waigaon and Lakdong, are low yielders and unsuitable for the main turmeric-growing regions, despite having high curcumin

concentrations. In addition to poor curcumin content and rhizome production, foliar disease incidence is a major challenge for agricultural improvement.

Rhizomes are the only vegetative propagation method used to grow turmeric. The production of flowers by turmeric is rather unusual. Even when flowers are present, very few seeds are produced. As a result, farmers of turmeric must use at least one healthy bud with rhizomes as seed. Moreover, the rate of multiplication of a single rhizome is quite slow. In a growing season of 8 to 10 months, only 10 to 15 lateral buds can form. Preserving rhizome seeds is a difficult undertaking.

The advancement of *in-vitro* technology has opened up new possibilities for improving crop plants. Many horticultural crops now use standardized tissue culture methods. However, investigations on crop enhancement can employ this technique. For regional types like Waigaon, Salem, and others, the *in-vitro* technique requirements must be standardized.

The micropropagation method would be crucial for rapid multiplication. The yield and quality of the crop must be improved due to the growing demand for it, both domestically and for export (Medhi and Bora, 1993). Therefore, the current study's objective was to confirm the rapid and efficient methods for surface sterilization of turmeric rhizome buds, efficient use of growth regulators for turmeric shoot and root proliferation and use of best media for hardening of the *in-vitro* grown plantlets.

Materials and Methods

Present study was conducted at Instructional farm, Department of Vegetable Science, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola and Plant Tissue Culture Laboratory, Biotechnology Centre, Department of Agril. Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, (M.S.) during the academic year 2021-22 and 2022-23.

Materials

- **Source of explant used and variety:** Healthy and disease-free rhizomes of the turmeric cultivar PDKV Waigaon used in this study were cultivated at Instructional farm, Department of Vegetable Science, Dr. PDKV, Akola.
- **Chemicals:** The salts for supplying major, minor elements and other ingredients required for the preparation of media were obtained from Himedia Laboratories Limited, Mumbai, India. The amino acids, vitamins and plant growth regulators used were of plant tissue culture grade from Himedia.
- **Glassware and Laboratory Equipment:** Thermostable, autoclavable and high grade borosil make glassware, various equipments and instruments were used as available at plant tissue culture laboratory of Dr. PDKV, Akola to carry out present work.
- **Culture Medium:** In the present study, Murashige and Skoog (MS) (Murashige and Skoog, 1962) media was used in combination with different plant hormones for *in-vitro* micro-propagation.

Methodology

The rhizomes with sprouting buds of 0.5 - 1.00 cm length were dissociated from dried scales and washed in running tap water to remove the adhering dirt and soil. Surface sterilized using liquid detergent labolene 5% and disinfected by Bavistin and $HgCl_2$ solutions at per the specified concentrations decided in the treatments followed by several washings by double distilled water to remove the traces of sterilant under the hood of laminar air flow cabinet. The sterilised buds were then inoculated on MS media fortified with different concentrations of growth regulators as per the treatment combinations. For every treatment, 80 turmeric rhizome buds (explants) were used. The airtight sealed culture bottles were then maintained in a culture room at $25\pm2^{o}C$ under 1500 to 2000 lux was provided by white fluorescent light with 14 hours of light and 10 hours of dark period.

The shoots formed *in-vitro* were isolated and subcultured on a same medium for multiplication and rooting. After the 5^{th} subculture cycle, individual shoots of length 3-4 cm were excised and transferred to rooting media containing different combinations of auxin (IBA) on half strength MS medium. The rooted plantlets of turmeric then removed from the glass bottles and then transferred to the portrays filled with different medias for hardening under greenhouse conditions.

The experiment was laid out in (CRD) Completely Randomized Design having 5 treatments and 4 replications of surface sterilization of turmeric explant, *in-vitro* shoot induction and hardening of *in-vitro* grown plantlets and 3 treatments and 7 replications of *in-vitro* root induction.

Results and Discussion

Surface sterilization of explants:

The data regarding the establishment of turmeric explant was found significantly influenced by different surface sterilization treatments having different

Table 1: Surface sterilization of explants.

Treatment number	Treatment details	Survival (%)	Contamination (%)
T_1	Control (DDW)	3.75(7.84)	96.25(82.15)
T_2	Bavistin 0.1% for 2 min + $HgCl_2$ 0.1% for 2 min	40.00(39.13)	60.00(50.83)
T_3	Bavistin 0.1% for 4 min + $HgCl_2$ 0.1% for 4 min	77.50(61.83)	22.50(28.13)
T_4	Bavistin 0.1% for 2 min + $HgCl_2$ 0.2% for 2 min	60.00(50.83)	40.00(39.14)
T_5	Bavistin 0.1% for 4 min + $HgCl_2$ 0.2% for 4 min	88.75(70.74)	11.25(19.22)
	'F' Test	**Sig.**	**Sig.**
	SE(m)±	**3.14**	**3.15**
	CD (1%)	**9.56**	**9.57**

Table 2: *In-vitro* shoot induction.

Treatment number	Treatment details	No. of shoots initiated per explant	Days for shoot initiation	Days required for shoot proliferation after shoot initiation	No of leaves per shoot
T_1	Control (MS media)	1.2	11.8	35.3	2.25
T_2	MS media + 2.0mg/l BAP+ 0.5mg/l NAA	2.0	10.7	31.5	3.15
T_3	MS media + 2.0mg/l BAP+ 1.0mg/l NAA	2.3	9.5	29.5	3.55
T_4	MS media + 4.0mg/l BAP+ 0.5mg/l NAA	1.6	10.9	33.3	2.90
T_5	MS media + 4.0mg/l BAP+ 1.0mg/l NAA	1.4	11.5	34.8	2.55
	'F' Test	**Sig.**	**Sig.**	**Sig.**	**Sig.**
	SE(m)±	**0.10**	**0.12**	**0.73**	**0.13**
	CD (1%)	**0.41**	**0.51**	**3.06**	**0.53**

concentrations of Bavistin and $HgCl_2$ with different time periods forming 5 treatments. The data presented in Table 1 revealed that maximum survival percentage (88.75 %) was found in treatment T_5 **(**Bavistin 0.1% for 4 min + $HgCl_2$ 0.2% for 4 min**)** which was found at par (77.50 %) with treatment T_3 (Bavistin 0.1% for 4 min + $HgCl_2$ 0.1% for 4 min). Whereas in the same treatment i.e. T_5 **(**Bavistin 0.1% for 4 min + $HgCl_2$ 0.2% for 4 min**)** the minimum contamination percentage (11.25 %) was found which was also found at par (22.50 %) to treatment T_3 (Bavistin 0.1% for 4 min + $HgCl_2$ 0.1% for 4 min). However, the minimum survival percentage (3.75 %) was found in treatment T_1 [Control (DDW)] whereas in the same treatment T_1 [Control (DDW)] maximum contamination percentage (96.25%) was found.

Fig. 1: Inoculation of turmeric explants in culture bottles.

Rhizome buds were found to be more effectively sterilized by higher $HgCl_2$ concentrations and longer exposure times than by lower concentrations and shorter exposure times. At modest dosages, the systemic fungicide bavistin (carbendazim) effectively lowers fungal contamination by penetrating deeply into cells without producing phytotoxicity. Because of the bleaching action of its two chloride atoms, which interact with proteins and kill organisms, mercuric chloride is poisonous. The percentage of contamination rises sharply as the

Fig. 2: Initiation of shoot.

Table 3: *In-vitro* root induction.

Treatment number	Treatment details	No. of roots per plant	No of days for root initiation
T_1	Control (½ MS media)	5.43	12.4
T_2	½ MS media + 1mg/l IBA	7.31	7.6
T_3	½ MS media + 2mg/l IBA	6.17	9.9
'F' Test	**Sig.**	**Sig.**	
SE(m)±	**0.15**	**0.16**	
CD (1%)	**0.62**	**0.64**	

concentration and duration of $HgCl_2$ and Bavistin exposure to the turmeric explants decrease. Tyagi (2004) and Sharma *et al.*, (2012) noted similar outcomes with turmeric.

In-vitro shoot induction

In present study, the surface sterilized rhizome buds of turmeric were inoculated in different media combinations containing growth regulators such as BAP & NAA of varied concentrations forming five different treatment combinations.

The data regarding the number of shoots initiated per explant was found significantly influenced by different concentrations of growth regulators such as BAP and NAA in MS media. The data presented in Table 2 shows that maximum number of shoots initiated per explant (2.3) were found in treatment combination T_3 (MS media + 2.0mg/l BAP+ 1.0mg/l NAA) which was found at par with treatment combination T_2 (MS media + 2.0mg/l BAP+ 0.5mg/l NAA), similarly in the same treatment combination number of leaves per shoot (3.55) were also found maximum and treatment T_2 (MS media + 2.0mg/l BAP+ 0.5mg/l NAA) was found at par to it.

Fig. 3: Shooting of turmeric plantlets.

Among the different concentrations of BAP and NAA in MS media, the significantly minimum days to

Fig. 4: Subculturing of turmeric plantlets.

Fig. 5: Rooting of turmeric plantlets.

Fig. 6: Hardening of turmeric plantlets.

Table 4: Hardening of *in-vitro* developed plantlets.

Treatment number	Treatment details	Survival of plantlets (%)	Plant height after one month of hardening (cm)	Number of leaves after one month of hardening
T_1	Control (Coco peat)	93.44(75.24)	11.74	4.70
T_2	Vermicompost	88.13(69.84)	7.96	3.90
T_3	Peatmoss	97.75(81.73)	15.22	5.50
T_4	Coco peat + Vermicompost (1:1)	95.69(78.08)	13.49	5.05
T_5	Vermicompost + Soilrite (1:1)	90.38(71.94)	9.74	4.25
	'F' Test	**Sig.**	**Sig.**	**Sig.**
	SE(m)±	**1.04**	**0.41**	**0.15**
	CD (1%)	**3.18**	**1.71**	**0.63**

shoot initiation (9.5) were found in treatment combination T_3 (MS media + 2.0mg/l BAP+ 1.0mg/l NAA) whereas the minimum days required for shoot proliferation after shoot initiation were also found in the same treatment combination, however it was found at par with the treatment T_2 (MS media + 2.0mg/l BAP+ 0.5mg/l NAA).

Shoot multiplication requires the presence of cytokinin and auxin, the combination of BAP and NAA is required for the maximum number of multiple shoots to be produced in *Curcuma longa*. Higher amounts of endogenous cytokinin in micro shoots cause cell multiplication, elongation, and division. Results similar to the above obtained results are also reported by Sit and Tiwari (1997), George *et al*., (1998) and Zapata *et al*., (2003), Ghosh *et al*., (2013) in Turmeric.

***In-vitro* root induction:**

In present study, the *in-vitro* grown plantlets of turmeric were transferred to the rooting media of different combinations containing growth regulator IBA in three different concentrations. Effective rooting treatments result in a highly rooted shoots and a well-developed root system.

The data regarding the number of roots per plant was found significantly influenced by different concentrations of growth regulator IBA. The data presented in Table 3 shows that significantly maximum number of roots per plant (7.31) were found in treatment combination T_2 (½ MS media + 1mg/l IBA). Similarly, significantly minimum number of days for root initiation (7.6) were also found in same treatment combination.

To improve root development *in-vitro*, auxin supplementation is necessary due to cytokinin inhibition. Auxin like IBA which is a rooting hormone when applied in proper quantity gives better results. It is a myth that high concentration of IBA reduces the days for root initiation rather it causes some reverse effects. Such kind of similar findings were also reported by Ranjan *et al*., (2001), Habiba *et al*., (2002) in banana and Ghosh *et al*., (2013) in turmeric.

Hardening of *in-vitro* developed plantlets

The data regarding the survival percentage of plantlets was found to be significantly influenced by different growing media. The data presented in Table 4 revealed that maximum survival percentage of plantlets (97.75 %), maximum plant height after one month of hardening (15.22 cm) and maximum number of leaves after one month of hardening (5.50) was found in treatment T_3 (Peatmoss). Whereas, minimum survival percentage of plantlets (88.13%), minimum plant height after one month of hardening (7.96 cm) and minimum number of leaves after one month of hardening (3.90) was found in treatment T_2 (Vermicompost).

As per the above results, plantlets thrived in peatmoss because of its high porosity, nutritional potential, and ability to retain water. For early *in-vitro* plant acclimation, peat moss is one of the most popular organic substrates. A natural and organic substance called peat moss aids young plants in retaining the right amount of moisture and essential nutrients during their early growth phases. This investigation supports earlier findings for turmeric by Naz *et al*., (2009) and Nayak *et al*., (2011).

Conclusion

Based on the results mentioned above, it is concluded that in case of surface sterilization of turmeric, the treatment of Bavistin 0.1% for 4 min and $HgCl_2$ 0.2% for 4 min had been found best. The full MS media with 2.0 mg/l BAP and 1.0 mg/l NAA was found to be most effective for *in-vitro* shooting of turmeric. The rooting in *in-vitro* plantlets of turmeric was reported best with ½ MS medium and 1 mg/l IBA. The peatmoss had been found superior in term of survival percent for hardening of the *in-vitro* grown plants of turmeric in different media.

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