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Starches as solidifiers for medicinal plant micropropagations and biomass accumulations

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Abstract: In plant tissue and cell culture studies, media compositions are one of the most important factors affecting the micropropagation procedure's efficiency. Micropropagation studies can be conducted for commercial productions of medicinal plants, and low-cost options always have significance in large-scale productions. Some media component substitutes have been studied to reduce production costs. Agar, the media solidifier, is one of the most expensive components of media compositions. In this study, corn and wheat starches were used as media solidifiers at 80 and 100 g/L concentrations, and their effects on plant growth (shoot elongations, shoot, node, and root numbers) and biomass accumulations (shoot and root fresh and dry weights) in *Lavandula officinalis* and *Digitalis purpurea* node cultures were reported. The results showed that starch type and their concentrations significantly affected plant growth. Maximum multiple shoot number was recorded in medium supplemented with 80 g/L starch and was 61.3% higher than the control. Biomass accumulations were not statistically significant; however, higher biomass accumulations were detected in starch-added media than in control. Consequently, corn and wheat starches can be used at these concentrations as a substitute for agar to induce multiple shoot formations in *L. officinalis* and *D. purpurea* node cultures.

Keywords: *Lavandula officinalis*; *Digitalis purpurea*; Starch; Micropropagation; Biomass accumulation

1 Introduction

Plant tissue culture techniques are one of the most popular and efficient ways of producing a high-yield biomass production of high-quality medicinal plants (Moraes et al. 2021). Plant tissues and plantlets can be grown homogenously under controlled conditions in laboratories without the effects of external conditions. High biomass accumulation is needed, as plant cells are biofactories that produce plant secondary metabolites, which are frequently utilized in the pharmaceutical, food, and textile industries. In order to increase biomass accumulation in plant tissue cultures, media compositions such as concentrations of macro- and microelements, growth regulators, and vitamins should be optimized first (Nartop 2018). The concentration of agar, which is essential for the solidification of culture media, is also essential. 6-7 g/L agar is generally added to the culture medium to prepare semi-solid media. However, the price of agar is the primary parameter that determines the cost of production, as it is the most expensive component of culture medium (Tyagi et al. 2007). In order to substitute agar, researchers use different and low-cost solidifiers such as herbal flours, starches, guar gum, isabgol-husk, and sago powder at different concentrations (Daut et al. 2011; Gour and Kant 2011; Özkaynak et al. 2016).

Digitalis purpurea L. (*Scrophulariaceae*) is a medicinal plant, and treatment with drugs based on *Digitalis* extracts is frequently used to strengthen cardiac diffusion and regulate heart rhythm. Digitoxin, digoxin, and lanatoside C are the cardiac glycosides extracted from the leaves of *D. purpurea* plants. The contents of these glycosides are affected when traditional agricultural methods obtain biomass production. Therefore, *in vitro* biomass production can be a better and more beneficial method to avoid the effects of climatic and soil conditions (Bourgaud et al. 2001; Roca-Perez et al. 2004).

Lavandula officinalis L. (*Lamiaceae*) (lavender) is an aromatic and medicinal plant used for its therapeutic properties since ancient times. The main components of the aerial parts and flowers are linalool, linalyl acetate,

monoterpenes, sesquiterpenes, flavonoids, and terpenoids. The extracts obtained from the plant have a sedative effect on the central nervous system and trigger memory and learning. Essential oil of lavender is also used for its antimicrobial, antibacterial, antitumor, anti-inflammatory, antihistaminic, and antidiabetic effects (Alnamer et al. 2012; Rabiei et al. 2014; Raisi et al. 2020).

Because of their valuable pharmacological activities, these two plants are always of interest to medicinal plant producers. Therefore, micropropagation of these valuable medicinal plants offers a standard production method, independent of climatic and soil conditions, which are important for commercial production, especially for the pharmaceutical industry. In our study, a low-cost micropropagation method, depending on starches as medium solidifiers, was investigated; nodes obtained from *in vitro*-grown *D. purpurea* and *L. officinalis* plantlets were cultivated in WPM media containing agar (control – 6 g/L), wheat and corn starches (80 and 100 g/L) to determine their physiologic effects on growth parameters and biomass production rates.

2 Materials and Method

2.1 Plant Material and Culture Conditions

Sterile *L. officinalis* and *D. purpurea* shoots, micropropagated from nodal explants and grown in WPM medium, were transferred to media given in Table 1. Node cultures of each species were cultivated for four weeks at 16 h photoperiod (4000 lux) and 24±1°C temperature to investigate the effects of wheat and corn starches on growth parameters and biomass production of *L. officinalis* and *D. purpurea*.

Table 1. Media composition used for *L. officinalis* and *D. purpurea* micropropagation.

Media	Basal Medium	Agar	Wheat Starch	Corn Starch	Sucrose	pH
Control	WPM	6 g/L	-	-	30 g/L	5.8
B80	WPM	-	80 g/L	-	30 g/L	5.8
B100	WPM	-	100 g/L	-	30 g/L	5.8
M80	WPM	-	-	80 g/L	30 g/L	5.8
M100	WPM	-	-	100 g/L	30 g/L	5.8

Woody plant medium (Lloyd and McCown 1980) (WPM) supplemented with 6 g/L agar and 30 g/L sucrose was used as basal medium. Other media were derived from WPM medium and contained different concentrations of wheat (80 and 100 g/L) and corn (80 and 100 g/L) starches. The pH of the media was adjusted to 5.8. The media were autoclaved at 121°C and a pressure of 1.2 kg/cm^2 for 15 mins before use.

In the fourth week of cultivation, all cultures were terminated. After plantlets were removed from culture vessels, their bottom parts and roots were cleared from agar with the help of a napkin. *In vitro*-grown shoots and roots were distinguished from each other, and their fresh weights were recorded. These shoots and roots were dried for seven days at room temperature (24±1°C), and their dry weights were recorded.

2.2 Growth Parameters

Growth parameters were determined as shoot elongations (SE), node numbers (NN), root numbers (RN), shoot numbers (SN), multiple shoot numbers (MSN), and biomass accumulations were specified as shoot fresh weights (SFW), shoot dry weights (SDW), root fresh weights (RFW) and root dry weights (RDW).

2.3 Statistical Analysis

The study was implemented in a factorial randomized plot design, and each experiment was repeated thrice. Fifteen explants were used for each replicate. Data were analyzed with ANOVA, and the post hoc tests were performed using the Tukey test at $p < 0.05$.

3 Results

All the node explants used in this study turned into plantlets, and their roots started to grow in the second week of the culture period. In Table 2, the results were given by means of the growth parameters and biomass accumulation.

SE was detected higher on *L. officinalis* (1.90 cm) than *D. purpurea* (1.17 cm). The highest SE was observed on *L. officinalis at* 3.21 cm in B100. The highest SE of *D. purpurea* was 1.87 in M100, which was 49.6% higher than the control. The interaction between plant and starch types was statistically significant (p=0.024), and the highest value was detected in *L. officinalis**wheat starch interaction (2.26 cm). Even though there was no statistical significance, the mean results of SE showed that wheat starch was found more beneficial than corn starch (Table 2c), and the use of 100 g/L starch had the highest SE (Table 2d), which was 50.4% higher than the control.

The highest NN was detected (9.62) on *L. officinalis* in the B100 medium, whereas the highest NN on *D. purpurea* was observed in the M80 medium (3.53) (Table 2a). The interaction between plant type and media was statistically significant (p=0.049), and the highest value was detected on *L. officinalis**100g (8.76). The effect of starches on mean NN was nearly the same: 4.43 in wheat starch and 4.44 in corn starch (Table 2c). Similar to SE, 100 g/L starch had the highest value in NN (5.16), which was 52.7% higher than the control (3.38) (Table 2d).

Table 2. (a) Shoot elongation (SE), node (NN), root (RN), shoot (SN) and multiple shoot numbers (MSN) of *L. officinalis* and *D. purpurea* in media supplemented with wheat and corn starch at 80 g/L and 100 g/L concentrations **(b)** Mean values of plant type **(c)** Mean values of starch type **(d)** Mean values of media.

Table 2a

Plant	Media	SE (cm)	NN	RN	SN	MSN
Lavandula officinalis	Control	1.23±0.22	5.64±1.54	1.05±0.41	1.73±0.22	16.30±3.92
	B80	2.36±0.34	6.71±0.94	1.16±0.10	2.07±0.33	48.89±14.59
	B100	3.21±0.43	9.62±0.43	1.67±0.17	2.82±0.21	26.67±1.29
	M80	1.99±0.55	6.93±1.36	2.24±0.33	2.38±0.55	21.48±7.53
	M100	1.38±0.73	7.89±0.79	3.13±0.44	1.87±0.61	17.04±4.13
Digitalis purpurea	Control	1.25±0.49	1.11±0.34	2.38±0.97	0.56±0.14	6.67±2.23
	B80	0.64±0.16	1.93±0.34	2.42±0.81	0.51±0.12	0.74±0.74
	B100	0.97±0.11	1.55±0.40	3.00±0.20	0.78±0.22	5.18±1.96
	M80	1.06±0.12	3.53±0.93	3.71±0.61	0.80±0.19	2.96±1.96
	M100	1.87±0.61	1.56±0.50	3.73±0.62	1.06±0.24	7.41±1.96

Table 2b

Plant Type	SE (cm)	NN	RN	SN	MSN
Lavandula officinalis	1.90 A	7.07 A	1.71 B	2.10 A	24.44
Digitalis purpurea	1.17 B	1.80 B	2.94 B	0.71 B	4.94
p	0.006	0.001	0.001	0.001	0.001

Table 2c

Starch Type	SE (cm)	NN	RN	SN	MSN
Wheat	1.60	4.43	1.94 B	1.41	17.41
Corn	1.46	4.44	2.71 A	1.40	11.98
p	0.553	0.976	0.031	0.950	0.090

Table 2d

Media	SE (cm)	NN	RN	SN	MSN
Control	1.23	3.38 B	1.71 B	1.15	11.48
80g	1.51	4.77 AB	2.38 AB	1.44	18.52
100g	1.85	5.16 A	2.88 A	1.63	14.07
p	0.139	0.026	0.028	0.098	0.189

Each value is the mean of 3 replications, each with 15 explant. Values within column followed by different letters are significantly different at the 0.05 level by the Tukey test.

RN was affected significantly by plant type (p=0.001), starch type (p=0.031), and media (p=0.028). The highest RN was observed in M100 (3.73) on *D. purpurea*, whereas the highest RN on *L. officinalis* (3.13) was detected in the same medium as well (Table 2a). The mean RN (Table 2c) was higher for corn starch (2.71) than wheat starch (1.94). The results showed that corn starch had better effects on root growth than wheat starch. Once again, similar to SE and NN, 100 g/L starch concentration had the highest RN value (2.22), which was 30% higher than the control (1.71) (Table 2d).

In SN, only plant type was statistically significant. The highest SN (2.82) was obtained in B100 on *L. officinalis*. The maximum SN on *D. purpurea*was 1.06 in M100 (Table 2a). As such, in NN, the effects of starches on mean SN were nearly the same: 1.41 in wheat starch and 1.40 in corn starch (Table 2c). 100 mg/L starch concentration was the highest (1.63) and 42% higher than the control (1.15) (Table 2d).

The interactions between plant type*media and plant type*starch type were found statistically significant for MSN (p=0.012 and p=0.034, respectively). The maximum values for MSNs were detected in *L. officinalis**80g interaction (35.19) and *L. officinalis**wheat starch interaction (30.62). However, in *D. purpurea*, corn starch was more beneficial than wheat starch (5.67 and 4.20, respectively). The mean values were higher in wheat than in corn starch (Table 2c). 80 g/L starch concentration (Table 2d) had the highest MSN and was 61.3% higher than the control.

Table 3: (a) Shoot fresh (SFW) and dry (SDW) weights, root fresh (RFW) and dry (RDW) weights of *L. officinalis* and *D. purpurea* in media supplemented with wheat and corn starch at 80 g/L and 100 g/L concentrations **(b)** Mean values of plant type **(c)** Mean values of starch type **(d)** Mean values of media.

Table 3a

Plant	Media	SFW (g)	SDW (g)	RFW (g)	RDW (g)
Lavandula officinalis	Control	1.14±0.30	0.18±0.04	0.52±0.21	0.03±0.01
	B80	0.76±0.04	0.16±0.02	0.17±0.06	0.03±0.01
	B100	0.71±0.02	0.13±0.01	0.31±0.15	0.04±0.01
	M80	0.83±0.19	0.16±0.04	0.58±0.09	0.09±0.01
	M100	0.80±0.08	0.14±0.01	0.61±0.05	0.11±0.01
Digitalis purpurea	Control	1.33±0.25	0.13±0.04	0.71±0.49	0.04±0.02
	B80	0.92±0.23	0.15±0.05	0.80±0.23	0.11±0.04
	B100	0.62±0.09	0.09±0.01	1.18±0.69	0.20±0.10
	M80	1.53±0.21	0.16±0.03	2.29±0.19	0.26±0.03
	M100	1.56±0.54	0.15±0.05	1.08±0.46	0.14±0.06

Table 3b

Plant Type	SFW (g)	SDW (g)	RFW (g)	RDW (g)
Lavandula officinalis	0.90 B	0.16	0.44 B	0.05 B
Digitalis purpurea	1.21 A	0.13	1.14 A	0.18 A
p	0.037	0.237	0.001	0.005

Table 3c

Starch Type	SFW (g)	SDW (g)	RFW (g)	RDW (g)
Wheat	0.91	0.14	0.61	0.08
Corn	1.20	0.15	0.97	0.15
p	0.059	0.473	0.078	0.075

Table 3d

Media	SFW (g)	SDW (g)	RFW (g)	RDW (g)
Control	1.23	0.15	0.63	0.05 B
80g	1.01	0.15	0.95	0.12 AB
100g	0.92	0.13	0.79	0.18 A
p	0.211	0.460	0.405	0.038

Each value is the mean of 3 replications, each with 15 explant. Values within the column followed by different letters are significantly different at the 0.05 level by the Tukey test.

In SFW, plant type was detected as statistically significant (p=0.037). The maximum SFW of *L. officinalis* was obtained in control (1.14 g), whereas 1.56 g of SFW was the maximum value for *D. purpurea* in M100 (Table 3a). In SDW, no statistical significance was detected amongst the parameters tested. The maximum SDWs for *L. officinalis* and *D. purpurea* were 0.18 g in control and 0.16 g in M80, respectively. Starch-type values were detected nearly the same (Table 3c).

RFW values were only statistically significant for plant type (p=0.001) (Table 3b). The maximum RFWs for *L. officinalis* and *D. purpurea* were were 0.61 g in M100 and 2.29 g in M80, respectively (Table 3a). The mean RFW values were higher in corn starch (0.97 g) than in wheat starch (0.61 g) (Table 3c). The highest mean RFW (0.95 g) was detected in 80g (Table 3d). RDW values were affected significantly by plant type (Table 3b) and media (p=0.005 and p=0.038, respectively). The maximum RDWs for *L. officinalis* and *D. purpurea* were 0.11 g in M100 and 0.26 g in M80, respectively. Corn starch had a higher biomass accumulation effect on mean RDW than wheat starch (0.15 g and 0.08 g, respectively) (Table 3b). The highest mean RDW was detected at 100g (0.18 g), which was 3.6 times higher than the control (0.05 g) (Table 3d).

4 Discussion

The first examinations of the starches' effects were done visually. Both corn and wheat starches at 80 and 100 g/L concentrations gave enough strength to the culture medium to place the node explants easily and support growth. Similarly, Ebile et al. (2022) reported no morphological or vitrification problems when banana shoots were cultivated in a medium supplemented with tapioca starch instead of agar.

SE values of *L. officinalis* were found to be higher in media supplemented with starches than in the control. In *D. purpurea*, only M100 values were higher than in the control. Wheat starch showed higher SEs on plantlets (Table 2c). The use of 100 g/L starch concentration had the highest SE. Corn and wheat starches have promoted elongations of shoots in both plant types in our study (Table 2d). Similarly, Özkaynak et al. (2016) reported the use of guar gum, a plant-based natural product, as a medium solidifier, and the results showed that plant height in *in vitro* cultures of *Solanum tuberosum* was enhanced from 5.9 cm to 7.76 cm when guar gum was added to MS medium at 12 g/L.

NN values showed an accordance with SE values as expected. The type of starch did not affect the NN. However, 100 g/L concentration had the highest NN and was 52.6% higher than the control. The number of nodes per explant was also enhanced in *S. tuberosum in vitro* cultures when 15 g/L guar gum was added to the MS medium (Özkaynak et al. 2016).

The number of roots in both plant types was higher than the control in starch-applied media. RNs of both plant types gradually increased as the starch concentration enhanced. RNs of corn starch-added media were higher than those of wheat starch-added media. This result showed that corn starch can be preferred in root culture studies, and 100 g/L concentration will benefit better root growth.

In micropropagation studies, one of the most critical parameters is the multiple shoot formation rate. High numbers of multiple shoot formations facilitate micropropagation and accelerate biomass accumulations. Therefore, the component that triggers the multiple-shoot formation should be specified. In our study, MSN values were affected by plant type*media and plant type*starch interactions. Interestingly, *L. officinalis* was mainly affected by wheat starch, whereas corn starch was more effective on *D. purpurea*. Daud et al. (2011) reported that potato starch was used effectively at 40-100 g/L concentrations in *Celosia* sp. stem cultures as the jelling agent, and the number of shoot regenerations was detected between 18.90 and 26.50. In some micropropagation studies, starches and agar were used together in culture media. The results showed that using lower amounts of agar (1-2 g/L) when combined with starches, enhanced the number of shoot regenerations (Karim et al. 2003; Mohamed et al. 2009; Daud et al. 2011). This result may be the consequence of the structure of the starches; they are carbohydrates and may act as carbon sources in culture conditions for plant explants for their better growth. Hence, different starches can be used alone or with agar in culture media to promote the rate of micropropagation via enhanced multiple-shoot formations.

Biomass accumulation is important for micropropagation studies, especially in producing valuable medicinal plants. Our study evaluated fresh and dry weights of shoots and roots. Although no statistical significance was detected in starch type and media experiments, corn starch had higher mean results among these four parameters. Hence, corn and wheat starches can be used instead of agar because of the lack of statistical significance between the parameters. However, different starches can be used to promote biomass accumulation. Özkaynak et al. (2016) reported that using 18 and 20 g/L guar gum in MS medium enhanced fresh weights in *S. tuberosum* from 0.71 g to 1.68 g and 1.12 g, respectively.

Different materials were used instead of agar for different plant and culture types. Jain and Babbar (2006) successfully used xanthan gum as a media solidifier in *Calliandra tweedii in vitro* cultures to obtain seed germination, caulogenesis and somatic embryogenesis. Interestingly, Deb and Pongener (2010) used polyurethane foam discs, chopped coconut and betel nut coirs and leaf litters instead of agar in order to obtain *in vitro* seed germination and plant regeneration of *Cymbidium aloi* and the best results were obtained from polyurethane foam. Moraes-Cerdeira et al (1995) reported that cotton fibers utilized for callus maintenance and shoot organogenesis of *Agrostis* and *Taxus* were found better than agar.

5 Conclusion

Using starches as media solidifiers is an essential low-cost option for micropropagation studies. The prizes of the starches are much lower than the agar prizes. Starches have been mainly used in nourishment. Therefore, they are easy to access. Moreover, their plant-based and non-toxic (edible) nature positively affects plant growth in *in vitro* conditions. They support plant explants in media as both media solidifiers and carbon sources. This study proved that corn and wheat starches did not show toxic or growth-inhibiting effects on *L. officinalis* and *D. purpurea* node cultures. Multiple shoot formations, which strongly affect the micropropagation rate, were promoted by 80 and 100 g starch additions. These results showed that more studies should be established about media solidifiers, which may be substituted for agar.

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Authors' contributions:

PN: Study conception, design, supervision, data analysis, tatistical analysis, literature review, manuscript writing, editing and laboratory experiments
MAF: Laboratory experiments
MT: Laboratory experiments

Conflict of interest disclosure:

The authors declare no conflict of interest.

References

Alnamer R, Alaoui K, Bouidida EH, Benjouad A, Cherrah Y (2012) Sedative and hypnotic activities of the methanolic and aqueous extracts of *Lavandula officinalis* from Morocco. *Adv Pharmacol Sci 5*. http://doi.org/10.1155/2012/270824.

Bourgaud F, Gravot A, Milesi S, Gontier E (2001) Production of Plant Secondary Metabolites: A Historical Perspective. *Plant Sci 161*:839-851. https://doi.org/10.1016/S0168-9452(01)00490-3

Daud N, Taha RM, Mohd NN, Alimon H (2011) Potential of alternative gelling agents in media for the *in vitro* micro-propagation of *Celosia* sp. *Int J Bot 7(2):*183-188. https://doi.org/10.3923/ijb.2011.183.188

Deb CR, Pongener A (2010) Search of alternative substratum for agar in plant tissue culture, Curr Sci 98(1):99-102.

Ebile PA, Opata J, Hegele S (2022) Evaluating suitable low-cost agar substitutes, clarity, stability, and toxicity for resource-poor countries' tissue culture media. In Vitro Cell Dev Biol – Plant 58:989-1001. https://doi.org/10.1007/s11627-022-10285-6.

Gour VS, Kant T (2011) Efficacy of low cost gelling agents and carbon source alternatives during *in vitro* rooting of *Balanites aegyptiaca* and *Phyllanthus emblica* microshoots. Tree Forest Sci Biotechnol *5(1):*58-60.

Jain R, Babbar SB (2006) Xanthan gum: an economical substitute for agar in plant tissue culture media. Plant Cell Rep 25:81–84. https://doi.org/10.1007/s00299-005-0039-8

Karim MZ, Amin MN, Azad MAK, Begum F, Rahman MM, Ahmad S, Alam R (2003) *In vitro* shoot multiplication of *Chrysanthemum morifolium* as affected by sucrose, agar and pH. Biotechnol 2, 115-120. https://doi.org/10.3923/biotech.2003.115.120

Lloyd GB, McCown BH (1980) Commercial-feasible micropropagation of mountain laurel- *Kalmia latifolia* by use of shoot- tip culture. Proc Int Plant Prop Soc 30:421–427.

Mohamed MAH, Alsadon AA, Al-Mohaidib MS (2009) Corn and potato starch as an alternative for *Solanum tuberosum* micropropagation. Afr J Biotechnol *8*:9199-9203. https://doi.org/10.5897/AJB09.638

Moraes RM, Cerdeira AL, Lourenco MV (2021) Using micropropagation to develop medicinal plants into crops. Molecules 26(6):1752.

Moraes-Cerdeira RM, Krans JV, McChesney JD, Pereira AMS, Franca SC (1995) Cotton fiber as a substitute for agar support in tissue culture. HortScience 30(5):1082-1083. https://doi.org/10.21273/HORTSCI.30.5.1082

Nartop P (2018) Engineering of Biomass Accumulation and Secondary Metabolite Production in Plant Cell and Tissue Cultures, Plant Metabolites and Regulation Under Environmental Stress, (eds.) Parvaiz Ahmad, Mohammad Abass Ahanger, Vijay Pratap Singh, Chapter 9, Elsevier, pp. 169-194, ISBN: 978-0-12-812689-9. https://doi.org/10.1016/B978-0-12-812689-9.00009-1

Özkaynak E, Yüksel F, Erüst N, Şimşek T (2016) Effect of using gelling agent guar gum and different sugar sources on potato micropropagation. Turk J Agr Nat Sci 3(4):249-254.

Rabiei Z, Rafieian-Kopaei M, Makhtari S, Alibabaei Z, Shahrani M (2014) The effect of pretreatment with different doses of *Lavandula officinalis* ethanolic extract on memory, learning and nociception. Biomed Aging Pathol 4(1):71-76. https://doi.org/10.1016/j.biomag.2013.10.006

Raisi A, Davoodi F, Afshar-Ghahremani S, Taheri S, Sharifi S, Adel M (2020) Evaluation of the anesthetic and tranquilizing effects of clove powder (*Syzygium aromaticum*) and lavender oil (*Lavandula officinalis*) in convict cichlid fish (*Cichlasoma nigrofasciata*). Iran J Vet Surg 15(1):1-7. https://doi.org/10.30500/IVSA.2019.193913.1191

Roca-Perez L, Boluda R, Gavidia I, Perez-Bermudez P (2004) Seasonal cardenolide production and Dop5βr gene expression in natural populations of *Digitalis obscura*. Phytochem 65:1869-1878. https://doi.org/10.1016/j.phytochem.2004.05.004

Tyagi RK, Agrawal A, Mahalakshmi C, Hussain Z, Tyagi H (2007) Low-cost media for *in vitro* conservation of turmeric (*Curcuma longa* L.) and genetic stability assessment using RAPD markers. In Vitro Cell Dev Biol– Plant 43:51-58.