Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7817

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2019) *7*(5), 131-137

Research Article

Indian Journal of Pure & Applied Biosciences

In vitro Propagation and Study of Thiourea in Effective Breakage of Bud Dormancy in White Yam (*Dioscorea rotundata* Poir.)

Parvathy Harikumar* and Sheela M. N.

Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala, India, 695017

*Corresponding Author E-mail: coolfrnzparu@gmail.com

Received: 7.09.2019 | Revised: 11.10.2019 | Accepted: 19.10.2019

ABSTRACT

Yams are one of the most important tuber crop grown in India. They are members of genus Dioscorea which produces tubers, bulbils or rhizomes having economic importance. They are consumed as staple food and are rich in starch and energy. The most important part of yam is tuber. Yam propagation is by seeds using conventional methods is slow and not adequate for rapid multiplication. In yam cultivation, the most important problem faced by farmers is the non availability of quality planting material, because part of the tuber itself is saved as planting material for next season. Quality of planting material should also be assured to ensure good sprouting and less contamination. In vitro propagation helps to avoid problems related to ex vitro. So the necessity of standardization of protocol for in vitro propagation is inevitable and attempted in the present study. It helps in mass propagation of bushy white yam and enhances the availability of planting materials to farmers. Yam tubers have long dormant period that restricts sprouting or germination faster. This leads to restricting supply and the rate of progress of crop improvement. Present study also focused on the application of thiourea which decreased the dormancy period progressively.

Keywords: *Yams, Tuber crops, Sprouting, In vitro propagation, Dormancy and thiourea.*

INTRODUCTION

Root and tuber crops are plants that are grown for their modified, thickened roots or stems, which generally develop underground (Bradshaw, 2010). The important tuber crops around the world are cassava, potato, sweet potato, yams and cocoyams. Current study is focused on one of the most important tuber crop called yam. Yams are considered as crops of ancient origin which were domesticated before 5000B.C. They are monocots, despite occasional evidence of the existence of a second cotyledon. They belong to the family dioscoreacea within the order dioscoreales (Ayensu & Coursey, 1972). The genus dioscorea is the largest genus of the family. The family includes ten genera and 650 species and are mainly tropical and subtropical and semi temperate in distribution.

Cite this article: Harikumar, P., & Sheela M.N. (2019). *In vitro* Propagation and Study of Thiourea in Effective Breakage of Bud Dormancy in White Yam (*Dioscorea rotundata* Poir.), *Ind. J. Pure App. Biosci. 7*(5), 131-137. doi: http://dx.doi.org/10.18782/2320-7051.7817

Most important species in economic point of view of the genus *Dioscorea* are *D. rotundata*, *D. alata*, *D. cayenensis*, *D. esculenta*, *D. dumetorum*, *D. bulbifera*, *D. trifida*, *D. opposita* and *D. japonica*. The most important yam on worldwide basis is *Dioscorea rotundata* Poir (White yam or white guinea yam) grown on larger area compared to other yam species (Waitt, 1961). All species of economic importance are tuberous.

The propagation of yam is via its tuber saved after harvesting. Tuber yield is drastically reduced by viral and nematode infections. Through infected tubers it is transmitted to the next generation and it also deteriorate the quality of the tuber. In yam cultivation, the most important problem faced by farmers is the non availability of quality planting material, because part of the tuber itself is saved as planting material for next season. Quality of planting material should also be assured to ensure good sprouting and less contamination. So the necessity of standardization of protocol for *in vitro* propagation is inevitable. Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods. It is used to multiply novel plants that have been genetically modified or bred through conventional plant breeding methods.

In vitro propagation is used to multiply novel plants for the production of planting material. *In vitro* plants can be used for the storage of breeding material with degenerated growth, to struggle against genetic erosion and it also provides phytosanitary qualities that are microbe free planting materials. Therefore *in vitro* propagation helps in mass propagation of bushy yam and enhances the availability of planting materials to farmers. Dormancy of tubers are very common in tuber crops, especially long dormant period is present for yam tubers. Effect of thiourea on dormancy breaking and yield of potato (*Solanum tuberosum* L.) minitubers marfona cv. in greenhouse was studied by Sardar et al. (2011). From the literature search, thiourea is identified as an effective dormancy breaker and the present study focused on reducing dormancy period of yam tubers and hence increasing the supply and faster crop improvement programme.

MATERIALS AND METHODS

Explant Preparation

Explants used were nodal segments, shoot tips and tuber bud sprouts of bushy white yam. Nodal segments and shoot tips were collected from field gene bank. Tuber sprouts were collected from harvested tubers stored in yam storage room ICAR- Central Tuber Crops Research Institute, Trivandrum. Collected nodal segments and shoot tips were initially washed thoroughly under tap water 3 to 4 times. After washing, cut the leaves of nodal segments carefully without damaging the node and tips. After cutting off the leaves, nodal segments and shoot tips were transferred to bottles having autoclaved water. Washed it once and transferred to another bottle having laboline solution, which have antibacterial action. The bottle were kept in shaker for 30 minutes. After this step, the explants were washed with autoclaved distilled water 3 to 4 times to remove soapy solution from the explants. The explants were transferred using autoclaved forceps to another bottle having fungicide solution called bavistin. The bottles were kept in shaker again for 30 minutes and rinsed it thoroughly using autoclaved distilled water till the bavistin solution diminishes. Took the clean and clear explants inside laminar air flow hood for inoculation

Tuber bud sprouts, after collecting it needed 5-6 times of rinsing with tap water and careful excision of outer layers of bud without damaging the meristem. After collecting buds, rinsed thoroughly with autoclaved distilled water 2 to 3 times and transferred to bottles having laboline solution and kept bottles at shaker for 1 hour to reduce chance for bacterial contamination. After that rinsed thoroughly with autoclaved distilled water till soapy solution disappears and transferred the buds to bottles having bavistin solution and kept it in shaker for 1.5 hr for the initiation of fungus free cultures. Further it is rinsed using

autoclaved distilled water till bavistin was washed out and took the neat and clear buds inside laminar air flow chamber for inoculation.

Surface sterilization standardization

The process was mainly done using mercuric chloride and sodium dichloroisocyanurate solution. For standardization, different percentage and treatment time were carried out for sodium dichloroisocyanurate as shown in (Table 1). Standardization of treatment time of mercuric chloride for yam is by giving 0.1% mercuric chloride treatment time of 5, 8, 10, 12, 15 minutes and the comparative study of both the chemicals in surface sterilization was analyzed.

Nutrient media formulation

The formulation of Murashige & Skoog's (1962) basal medium (MS) is used in the propagation study of bushy white yams. MS basal salt mixture with vitamins and without calcium chloride was used. On preparation, the readymade pack of 1L MS medium was mixed thoroughly with sucrose (30g/l), which was weighed separately. After that pH of the medium was checked and confirmed between 5.6 to 5.8.Then 8 g of agar is added to the medium with activated charcoal and heated it till bubble formation. Filtered it and poured to sterile test tubes, capped it using foil paper and stored for 2 days to analyze any contamination and then used for inoculation. For the effective study and standardization, the formulation of the medium was modified and given in table 2.

Inoculation

Surface sterilized explants were inoculated in autoclaved medium inside laminar air flow using sterilized forceps. The inoculated tubes were capped using alcohol treated foil papers.

Culture conditions

All the cultures were kept at 25+-1^{0}C under 12 hour of photoperiod and at 70% to 80% relative humidity. Culture tubes were examined daily and data collected.

Thiourea treatment for breaking bud dormancy

Thiourea solution was prepared at concentration of 20g/l and stored in amber colored bottles. Tubers of *Dioscorea rotundata* after harvest were collected. From this collection few tubers of white yam dwarf variety Sreedhanya was selected and washed under tap water for several times to remove soil and dust from the tubers. After drying, tubers were cut into head, middle and tail portions carefully. Labeled each portion with tag and treated each part of tubers according to the tests listed on (Table 3). Took the cut tubers with forceps and completely immersed in 100ml thiourea solution of various concentration. After the listed time in different tests transferred the treated tubers to soil filled pots in the net house .Six treated tuber parts were planted on each pot and pots were labeled with date, test number and sample name. Days taken for sprouting of tubers were recorded and documented. Control was kept for comparison.

RESULT AND DISCUSSION

Five different media were used for the study and identified MS medium without hormones enhanced sprouting and shoot growth. MS medium with 2mg kinetin enhanced rooting compared to other media were shown in Table 4 and Figure 1. Sodium dichloroisocyanurate solution of 2 per cent for 15 minutes treatment found optimum for surface sterilization and got 70% recovery without any microbial contamination. Other concentrations lead to microbial contamination with treatment time less than 15 minutes. Increased treatment time with different concentration of sodium dichloroisocyanurate solution caused browning of explants, especially shoot tips. Mercuric chloride of 0.1 per cent for 8 minutes gave optimum surface sterilization results with 80 per cent recovery. Below 8 minutes treatment time caused increase in rate of contamination and low per cent recovery and more than 8 minutes treatment caused browning of shoot tips and tuber sprouts. Investigation on thiourea treatment showed that increasing concentration of thiourea progressively decline sprouting time as shown in fig 2. The thiourea treated plants in net house is shown in Figure 3.

Major problem faced during micropropagation is the delay of sprouting and contamination due to systemic microbes. The present finding was in conformity with the reports of Das et al. (2013) in greater Yam. Many workers reported *in vitro* propagation in several crops including yams. Mahesh et al. (2010) worked with *Dioscorea wightii* and propagated the plant using nodal segment as explants. BA and kinetin was used for the multiplication of nodal segment. Callus initiation was observed in MS medium supplemented with 0.15-1.75µM BA, 0.75-5.0µM kinetin, 0.15-0.30 µM 2iP and shoot formation was observed in all growth regulators tested in BA, Kinetin and 2iP. Chen et al. (2003) developed a protocol for rapid *in vitro* propagation of *D. zingiberensis* using stem as explants. Medium supplemented with 4.4µM BAP+1.1 µM NAA produced shoots on nodal segments within 20 days. Callus formed on MS +8.9 µM BA+ 5.4 µM NAA in 30 days, 22.2 µM BAP and 1.1 µM NAA regenerated shoot from callus and for rooting 4.9 µM IBA was used. Ovono et al. (2010) had studied the tuber formation and development of *Dioscorea cayenensis–Dioscorea rotundata* complex under *in vitro* conditions.

Effects of synthetic hormone substitutes and genotypes on rooting and mini tuber production of vines cuttings obtained from white yam (*Dioscorea rotundata*, Poir) was studied and the result showed that rice straw ash and neem leaf powder could serve as substitute to IBA hormone as root promoting substance in yam vine cuttings using carbonized rice husk as planting medium. For soaking method, 5% rice straw ash enhanced rooting of vine cuttings of the genotypes tested. Coconut water at 5% dilution in water was also found useful as a root - promoting substance for vine cuttings (Agele et al., 2010). Effects of storage conditions on sprouting of microtubers of yam (Dioscorea cayenensis–*D. rotundata* complex) was analyzed and found that conditions like the storage duration, the conditions of humidity, temperature and luminosity during storage and of the size of microtubers affects the sprouting rate. Dormancy phase was observed after 4 weeks of storage, its duration was between 20 and 28 weeks and storage temperature of 25^{0}C permitted a quicker sprouting than 18 ^{0}C (Ovono et al., 2010). However initial establishment of the explants is very slow and took 82-115 days. Hence further research is needed for hastening the *in vitro* establishment in white yam. Present investigation on thiourea for breaking dormancy also showed a slow progressiveness of sprouting on increasing concentration.

Table 1: Various treatments using sodium dichloroisocyanurate solution for surface sterilization

SL. No	Conc. of sodium dichloroisocyanurate	Treatment time (minutes)
1	2%	5, 10, 15, 20
2	1%	5, 10, 15, 20
3	0.5%	5, 10, 15, 20

Table 2: Composition of nutrient media formulation

SL. No	Media Code	Media Composition
1	MS	MS+ Sucrose 30g$^{L^{-1}}$ + Agar 8g$^{L^{-1}}$+ Charcoal 1g$^{L^{-1}}$
2	MSK1	MS+ Sucrose 30g$^{L^{-1}}$ + Agar 8g$^{L^{-1}}$+ Charcoal 1g$^{L^{-1}}$ + 1mg kinetin
3	MSK2N	MS+ Sucrose 30g$^{L^{-1}}$ + Agar 8g$^{L^{-1}}$+ Charcoal 1g$^{L^{-1}}$ + 2mg kinetin +.5mg NAA
4	MSK2	MS+ Sucrose 30g$^{L^{-1}}$ + Agar 8g$^{L^{-1}}$+ Charcoal 1g$^{L^{-1}}$+ 2mg kinetin
5	MSBN	MS+ Sucrose 30g$^{L^{-1}}$ + Agar 8g$^{L^{-1}}$+ Charcoal 1g$^{L^{-1}}$ + 2mg BAP + .5mg NAA

Table 3: Various experimental protocols performed for thiourea treatment of tubers

No of Tests	Conc. of thiourea	Amt of thiourea sol	Dist. water	Time of incubation
T_1	20g/l	100ml	0	60 min
T_2	20g/l	100ml	0	30 min
T_3	10g/l	50ml	50ml	60 min
T_4	10g/l	50ml	50ml	30 min
T_5	5g/l	25ml	75ml	60 min
T_6	5g/l	25ml	75ml	30 min
T_7	1g/l	5 ml	95ml	60 min
T_8	1g/l	5 ml	95 ml	30 min
T_9	Not used	0	100ml	60 min
T_{10}	Not used	0	100ml	30 min
Control	Not used	0	0	0

Table 4: Standardization of MS medium for in vitro propagation of white yam

Sample	Explants used	Media code	Surface sterilization method	No of days taken for sprouting	Shooting	Rooting
V1	Nodal cuttings	MS	Sodium dichloroisocyanurate (2% for 15 minutes)	115days	+ (3.8 cm)	+
V1	Nodal cuttings (from initial sprout)	MS	Sodium dichloroisocyanurate (2% for 15 minutes)	82days	+++++ (4.5 cm growth with 5-6 no of large dark green leaves)	+++
V1	Shoot tip	MSK2	Sodium dichloroisocyanurate (2% for 15 minutes)	112days	++ (2.1cm growth with one large light green leaf)	++++++
V1	Tuber sprout	MS	0.1% mercuric Chloride for 8 minutes	30 days	Initial growth of Callus	

Fig. 1: *In vitro* propagation of dwarf white yam

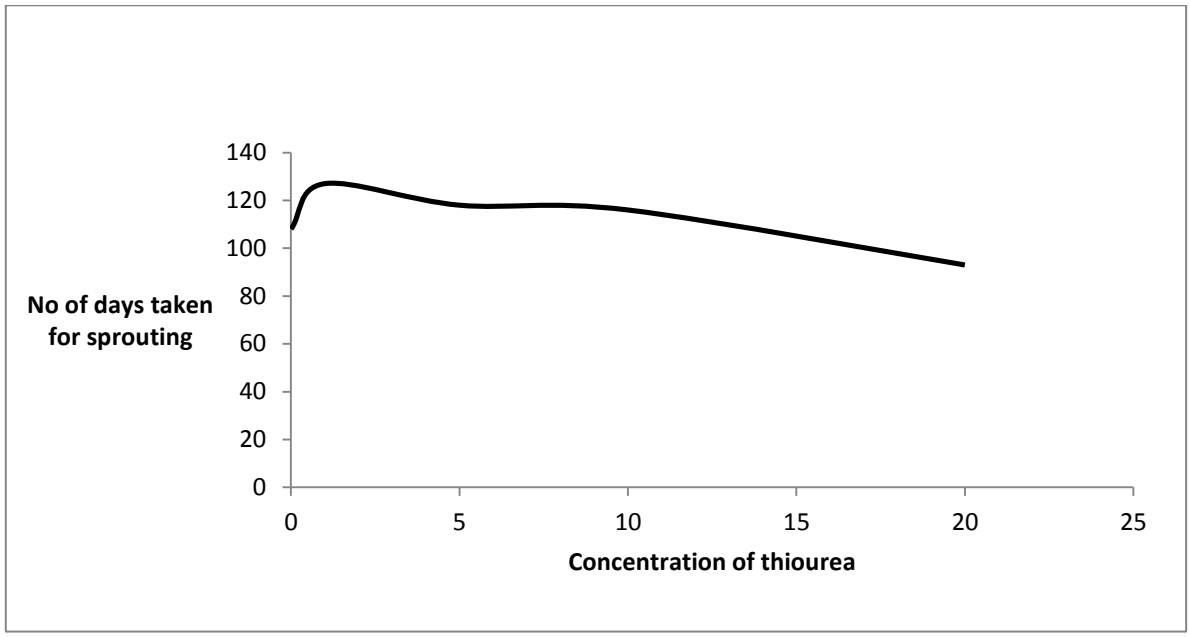

Fig. 2: Relationship between thiourea concentration and number of days for sprouting

Fig. 3: Thiourea treated plants in net house

CONCLUSIONS

From the present study it is identified that MS medium without hormones enhanced sprouting and shoot growth of white bushy yam. MS medium with 2 mg kinetin enhanced rooting compared to other media. Sodium dichloroisocyanurate solution of 2 per cent for 15 minutes treatment found optimum for surface sterilization and got 70% recovery without any microbial contamination. Mercuric chloride of 0.1 per cent for 8 minutes gave optimum surface sterilization results with 80 per cent recovery. However initial establishment of the explants is very slow and took 82-115 days. Hence further research is needed for hastening the *in vitro* establishment in white yam. Present investigation on thiourea for breaking dormancy also showed a slow progressiveness of sprouting on increasing concentration.

REFERENCES

Agele, S. O., Ayankanmi, G. T., & Kikuno, H. (2010). Effects of synthetic hormone substitutes and genotypes on rooting and mini tuber production of vines cuttings obtained from white yam (*Dioscorea rotundata* Poir). *Afr. J. Biotechnol. 9*, 4714 - 4724.

Ayensu, E.S., & Coursey, D. G. (1972). Guniea yams. The botany, ethnobotany, use and possible future of yams in West Africa. *Econ. Bot. 26*: 301-318.

Bradshaw, J. E. (2010). Root and Tuber Crops. Handbook of Plant Breeding, *7*, Springer Verlag, London.

Chen, Y., Fan, J., Yi, F., Luo, Z., & Fu, Y. (2003). Rapid clonal propagation of *Dioscorea zingiberensis*. *Plant Cell Tissue Organ Cult*. *73*, 75-80.

Das, S., Dutta, M., & Choudhury, P.B. (2013). MazumderIn *vitro* propagation of genus *Dioscorea* - a critical review, *Asian J. Pharmaceut. Clin. Res. 6*, 26-30

Germchi, S., Behroozi, F.G., & Badri, S. (2011). Effect of thiourea on dormancy breaking and yield of potato (*Solanum tuberosum* L.) minitubers Marfona cv. in greenhouse. In: Proceedings of the International Conference on Environmental and Agriculture Engineering (IPCBEE), *15*, IACSIT Press, Singapore *(19) (PDF) Thiourea: A Molecule with Immense Biological Significance in Plants*.

Mahesh, R., Muthuchelian, K., Maridass, M., & Raju, G. (2010). *In vitro* propagation of wild yam (*Dioscorea wightii*) through nodal cultures. *Int. J. of Bio. Technol*. *1*, 111-113.

Ovono, O. P., Kevers, C., & Dommes, J. (2010a). *In Vitro* Preservation of Yam (*Dioscorea cayenensis – D. rotundata* complex) for a Better Use of Genetic Resources. *Not. Bot. Hort. Agrobot. Cluj*. *38*, 141-146.

Ovono, O. P., Kevers, C., & Dommes, J. 2010b. Tuber formation and development of *Dioscorea cayenensis–Dioscorea rotundata* complex *in vitro* effect of polyamines. *In vitro Cell. Dev. Biol. 46*, 81–88.

Waitt, A. W. (1961). Key to some Nigerian yam varieties. *Memo Fed. Dept. Agric. Res*., Nigeria.