Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.3, No.1, 2013

Regeneration of Plantlets from various Explants of Tetraploid watermelon

*Misbah Rasheed[1,2], Muhammad Jafar Jaskani[1], Mehwish Rasheed[1], Muhammad Shahid Iqbal[1,4], Syed Zia-Ul-Hasan[3,4], Rizwan Rafique[1,2], Salman Mushtaq[1] and Majid Iqbal[1]

[1]University of Agriculture, Faisalabad, Pakistan
[2]Department of Agricultural Extension, Punjab, Pakistan
[3]Ayub Agricultural Research Institute, Faisalabad, Pakistan
[4]PMAS-Arid Agriculture University, Rawalpindi, Pakistan.
*Corresponding author: anamvirgo@gmail.com

Abstract
Micropropagation of tetraploid watermelon is important to cope with high cost of seed. Seeds of tetraploid watermelon were grown in vitro to raise seedlings. Hypocotyl and cotyledonary explants and media supplemented with plant growth regulators (BAP and NAA) was explored for callus induction and organogenesis. Data was collected for callus, shoot and root induction. Maximum callus induction was observed at BAP 5 mgL^{-1} (76.66%) from cotyledon and (73.33%) from hypocotyls explant. The callus induced from different explants was sub-cultured on the shoot regeneration medium. Higher shoot induction (96.66%) was observed from cotyledon and hypocotyl explant (76.66%) on MS + 1.0 mgL^{-1} BAP + 0.2 mgL^{-1} NAA with maximum number (6.3) of shoot per explant and average shoot length 4.5 cm. Among different types (NAA and IAA) and concentrations (0, 0.1, 0.3, 0.7, 1.0 mgL^{-1}) of auxins investigated for root induction, maximum frequency of rooting was observed in 0.1 mgL^{-1} NAA while no root formation was observed at higher levels of auxin (1.0 mgL^{-1}). Similarly in case of number of roots per shoot maximum root (4.3) was obtained on MS medium supplemented with 0.1 mgL^{-1} NAA.
Key Words: Regeneration, Explant, Tetraploid, Water Mellon

1. Introduction:

Watermelon (Citrullus lanatus Thumb.) belongs to the family Cucurbitaceae and is an important vegetable crop world-wide with 931,173,368 tons annual production (Anonymous, 2008). China is the biggest producer of watermelon in the world. The area under cultivation is about 2,162,456 ha and production is 67,203,275 MT. The area under watermelon cultivation in Pakistan is 27,939 ha and production is 411,996 MT (Anonymous, 2008).

Watermelon is important throughout the world because of its high vitamin and nutrient content. Watermelon flesh is also rich in lycopene, a potent antioxidant that has been shown to reduce human risk to cancer of the prostrate, pancreas and stomach (Garster, 1997). It is a juicy fruit and act as body coolant. It is a good source of fiber, which helps to reduce cholesterol and is important for keeping our digestive tract operating properly (Sultana *et al.*, 2004). The nutritive value of seed is due to its oil and protein contents. On an average, red flesh contain 55 percent of fruit weight. Because of high sugar contents, juice and attractive red color; the fruit is an ideal material for processing and product development.

Excessive seed number in watermelon fruit is fast becoming unacceptable in international markets. Seedless cultivars have been available for over 60 years (Kihara, 1951) and are becoming more prevalent (Lucier & Lin, 2001). Seedless cultivars are in high demand by consumer not only because of their fruits are seedless but also because their fruits are sweeter than fruits from diploid, seeded cultivars (Marr & Gast, 1991).

Seedless watermelons are triploid (2n=3x=33) hybrids. So to get triploid hybrids we require tetraploid (female) and diploid (male) plants. Because tetraploids exhibit reduced fertility. First generation tetraploids may produce only 5–20 seeds per fruit and limited fruit production per plant (maximum of 3–4 fruits per plant with seeds) and as many as 10 years may be required to produce the quantity of seed needed to satisfy the commercial demand for new triploid seed (Compton *et al.*, 2004). Tetraploids are commonly sterile and yield lower number of seeds per fruit (Jaskani et al, 2005). Hence tissue culture for mass clonal propagation of tetraploid watermelon could be a potential alternative.

The objective of the present research is to test different explants, cultural conditions and plant growth regulators (PGRs) for the development of a suitable protocol for mass clonal propagation of tetraploid watermelon.

2. Material and Methods

The present research was carried out at Plant Tissue Culture Cell, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Seeds of tetraploid watermelon cultivar viz. Sugar baby were soaked in double distilled water for one hour. These seeds were decoated and disinfected with 70% ethanol (v/v) for five minutes and then in 5% sodium hypochlorite (v/v) for three minutes followed by two to three rinses with double distilled water. After removing seed coat, the seeds were cultured on Murashige & Skoog (1962) medium to grow seedlings so as to get explants (hypocotyl and cotyledonry leaves) from these.

For callus induction, the explants were cultured on MS medium supplemented with BAP (0, 3, 5 and 10 mg/L). The cultures were maintained at 25°C under dark conditions and with 16 h photoperiod. For shoot initiation MS medium was fortified with BAP (0, 0.5, 1.0, and 2.0 mg/L) and NAA (0.2 and 0.5 mg/L) in combination. For root induction, regenerated shoots were shifted in MS medium added with NAA (0, 0.1, 0.3, 0.7 and 1.0mg/L) and IBA (0, 0.1, 0.3, 0.7 and 1.0mg/L). The cultures were maintained at 25°C with 16 h photoperiod.

3. Results and Discussion

Seeds of tetraploid watermelon cultivar viz. Sugar baby were decoated and cultured on MS medium which started germination in vitro in 5-20 days and germination increased with time. After 20 days of culture, maximum seed germination (86.66%) was observed. Explants (hypocotyl and cotyledonry leaves) wereobtained from these seedlings. MS medium was supplemented with different concentrations of BAP (0, 3, 5 and 10 mgL^{-1}) to observe the callus induction and results showed that maximum mean value for callus induction was observed at 5 mgL^{-1} as shown in table 1. Maximum callus induction was observed when cotyledons were cultured in MS medium supplemented with BAP 5 mgL^{-1} follow by 10 mg L^{-1} (43.33%) and minimum callus induction (23.33%) was observed at 3 mgL^{-1}.

Callus induction percentage from hypocotyl culture on MS Medium supplemented with different concentrations of BAP revealed that significantly higher amount of callus (73.33%) was induced at 5mgL^{-1} of BAP followed by BAP (10 mgL^{-1}). Lowest callus induction (10%) was depicted on BAP (3 mgL^{-1}) with over all mean value (35.83) for callus induction (%) from cotyledon and (26.67) from hypocotyls explant as shown in table 1. El-Zeiny (2007) also reported that shoot and callus together was obtained from shoot tip explants in cucumber when cultured on media supplemented with high levels of cytokinin i-e BA 4.0 mgL^{-1}. Berg *et al.* (1997) reported BAP as sole plant growth regulator for good texture callus development. Selvaraj *et al.* (2007) produced organogenic, green, compact callus from 86.2% of responding cotyledon explants on NAA (2.69 mM) and BA (4.44 mM) combination in cucumber.

Maximum shoot induction (96.66%) was observed from callus on MS medium supplemented with 1.0 mgL^{-1} BAP + 0.2 mgL^{-1} NAA followed by 1.0 mgL^{-1} BAP alone (93.33%) where as the lowest shoot development was observed in hypocotyl explant (36.66%) on MS medium supplemented with 2.0 mgL^{-1} BAP + 0.5 mgL^{-1} NAA. Among treatments in both explants the combination of BAP and NAA at the rate of 1.0 mgL^{-1} and 0.2 mgL^{-1} respectively added in MS medium show maximum shoot induction percentage and further increase decreased shoot induction (56.66%) suggesting 1.0 mgL^{-1} as optimum level of BAP from shoot induction in watermelon as shown in table 2.

The induction of multiple shoots in cotyledon explants varied with concentration and the type of plant growth regulators. Regeneration of adventitious shoots has been reported from a wide range of diploid and tetraploid watermelon cultivars (Srivastava *et al.*, 1989; Dong & Jia, 1991; Compton & Gray, 1993). In all reports, cotyledons of in vitro-germinated seedlings were the best source of explants. Two- to five-day-old seedlings with their cotyledons in close contact have displayed the greatest organogenic competence (Compton & Gray, 1993; Choi *et al.*, 1994). Most of the reports on cotyledon culture in cucurbits describe the direct shoot regeneration without any callus phase as in the case of cucumber (Cucumis sativus L.) (Gambley & Dodd, 1990; Handley & Chambliss, 1979) and muskmelon (Cucumis melo) (Niedz *et al.*, 1989). But in the present study we obtained multiple shoots through callus regeneration from cotyledons. In the present study cytokinin alone or a combination of auxin and cytokininwas essential for the regeneration of the calli. BAP alone or in combination with NAA gave organogenesis. Similar results were reported in several cucurbits like C. melo (Niedz *et al.*, 1989), Citrullus vulgaris (Dong & Jia, 1991) as well as other systems like Picea abies (Bornmant, 1983) and Stylosanthes guianensis (Mroginski & Kartha, 1981).

Our results are in conformity with the findings of Sultana & Bari (2003). They observed that 1.0 mgL^{-1} BA + 0.2 mgL^{-1} NAA was best for production of multiple shoots in watermelon. The auxin to cytokinins ratio represents an important signal in the formation of cell phenotype since auxins are capable of imitating cell division they are involved in the formation of meristems giving rise to either unorganized tissue or defined organs (George *et al.*, 2008 & Hasan *et al.*, 2010). The results of the present investigation also agree with the findings of Hoque *et al.* (1995).They found that a combination of 1.5 mgL^{-1} and 0.1 mgL^{-1} was more suitable for adventitious shoot formation in teasle gourd. Nishibayashi *et al.* (1996),

Raharjo *et al.* (1996) reported adventitious shoot formation in cucumber using hypocotyl explants in IAA, NAA and BA combinations. Selvaraj *et al.* (2006) also reported maximum adventitious shoot production (25 shoots/explant) in a BA/zeatin combination for hypocotyl explants of cucumber. Abdul-Awal *et al.* (2005) also reported 100% shoot induction when shoot tips of pointed gourd were cultured on MS medium containing BA (1.0 mgL^{-1}) and BA (1.0 mgL^{-1}) in combination with NAA (0.2 mgL^{-1}). Thomas & Sreejesh (2004) recorded 95% cultures showed shoot regeneration on MS medium fortified with BAP (4.0 µM) + NAA (0.2 µM) with 10.6 shoots per culture 5 weeks after callus culture.

Table 1: Effect of BAP on callus induction (%) from different explants of watermelon

MS + BAP (mgL^{-1})	Explant		Mean
	Cytoledon	Hypocotyl	
0	0.00 e	0.00 e	0.00 d
3	23.33 c	10.00 d	16.67 c
5	76.67 a	73.33 a	75.00 a
10	43.33 b	23.33 c	33.33 b
Mean	35.83 a	26.67 b	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Table 2: Effect of BAP and NAA on shoot induction (%) from different explants of watermelon

MS + BAP + NAA mgL^{-1}	Shoot induction (%)		No. of shoots per explant	
	Hypocotyl	Cotyledon	Hypocotyl	Cotyledon
Control	0.00±0.00 h	0.00±0.00 h	0.00±0.00	0.00±0.00
MS + 0.5 + 0	43.33±3.33 fg	56.67±3.33 cde	2.67±0.67	2.67±0.33
MS + 1.0 + 0	50.00±5.77 ef	66.67±3.33 bc	3.33±0.33	3.00±0.00
MS + 2.0 + 0	60.00±0.00 cde	93.33±3.33 a	5.33±0.33	4.33±0.33
MS + 0.5 + 0.2	66.67±3.33 bc	43.33±3.33 fg	2.67±0.33	2.33±0.33

MS + 0.5 + 0.5	50.00±5.77 ef	66.67±3.33 bc	3.33±0.33	3.33±0.33
MS + 1.0 + 0.2	76.67±3.33 b	96.67±3.33 a	6.33±0.33	5.67±0.33
MS + 1.0 + 0.5	53.33±3.33 def	90.00±5.77 a	4.33±0.33	3.00±0.58
MS + 2.0 + 0.2	50.00±5.77 ef	56.67±3.33 cde	3.00±0.58	2.00±0.58
MS + 2.0 + 0.5	36.67±3.33 g	63.33±3.33 cd	3.00±0.00	1.33±0.33
Mean	48.67±3.77 B	63.33±5.08 A	3.40±0.32 a	2.77±0.29 b

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Significantly (probability <0.05) higher no. of shoots (6.3) were observed on 1.0 mgL^{-1} BAP + 0.2 mgL^{-1} NAA in calli derived from cotyledon followed by (5.6) shoots in hypocotyls explant. Further increase in BAP 2 mgL^{-1} 0.2 mgL^{-1} NAA showed decrease in no. of shoots (3) in cotyledon explant and (2) in hypocotyl explant as shown in table 2b. and also in the onset and maintenance of the process of cell division

The above results of shoot induction percentage are in conformity with the findings of Pirinc *et al.* (2003). They found that mean number of shoots per explant and mean shoot length were highest when the medium was supplemented with BA (0.5 and 1.0 mgL^{-1}) and also in accordance with the findings of Compton and Gray (1993) who stated that the shoot regeneration of watermelon cotyledons was best on MS medium with BA (1 or 2 mgL^{-1}).

The shoots induced from cotyledon leaf and hypocotyl regenerated less roots on control and higher level of NAA (1.0 mgL^{-1}). The highest root regeneration percentage was generated on the lower levels of NAA (0.1 and 0.3 mgL^{-1}). The application of auxins to micro propagated shoots seems to intensify the root number by mounting the endogenous contents of enzymes (Asghar *et al.*, 2011 & Rafique *et al.*, 2012). The root regeneration was decreased with increase in concentration of NAA. Lowest root regeneration frequency was observed on higher level of auxins.

Table 3 showed mean values of root induction (%) on different levels of NAA and IAA Maximum root induction (96.66%) was observed when all individual shoots separated from multiple shoot stocks were directly cultured on MS medium supplemented with 0.1 mgL^{-1} NAA followed by 0.3 mgL^{-1} NAA (73.33%) whereas the lowest root development was observed (40%) when shoots were cultured on MS medium supplemented with 1.0 mgL^{-1} IBA.

Similar results were also observed by Sultana *et al.* (2004). They found that among different concentration of auxins, NAA showed comparatively better response than IBA and IAA for producing roots. Best rooting was observed with half strength of MS medium supplemented with 0.1 mgL^{-1} NAA and highest

number roots per microcuttings were 4.65±0.35. The findings were in agreement with those observed in other plant species such as caphaelis ipecacuanha, Plantago ovat.

Chaturvedi & Bhatnagar (2001) also concluded that for the initiation of roots, 3.0 cm long shoots were individually transferred to MS medium supplemented with NAA (0.5 mM). In this treatment 91.5% of shoots produced roots directly at the base within 6 d, with an average of six roots per explant. Significantly (probability <0.05) higher no. of roots (4) were observed on MS medium fortified with 0.1 mgL^{-1} NAA expressed in Table 3. Further increase in NAA 0.3 mgL^{-1} showed decrease in no. of roots (2.6) per explant and the minimum roots was observed in 0.3 mgL^{-1} IAA. Similar results were also observed by Sultana *et al.* (2004) that among different concentration of auxins, NAA showed comparatively better response than IBA and IAA for producing roots in watermelon. Best rooting was observed with half strength of MS medium supplemented with 0.1 mgL^{-1} NAA and highest number roots per microcuttings were 4.65±0.35.

Table 3: Effect of NAA and IAA on root induction (%) in microshoots of watermelon

Auxin's concentration (mgL^{-1})	Root induction (%)		No. of roots per microshoot	
	NAA	IAA	NAA	IAA
Control	0.00±0.00 e	0.00±0.00 e	0.00±0.00 d	0.00±0.00 d
0.1	96.67±3.33 a	63.33±3.33 c	4.33±0.33 a	2.33±0.33 bc
0.3	73.33±6.67 b	40.00±5.77 d	2.67±0.33 b	2.00±0.58 bc
0.7	56.67±3.33 c	0.00±0.00 e	2.00±0.00 bc	1.67±0.33 c
1.0	0.00±0.00 e	0.00±0.00 e	0.00±0.00 d	0.00±0.00 d
Mean	45.33±10.55 a	20.67±7.14 b	1.80±0.45 a	1.20±0.30 b

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Well-developed plants were shifted to small pots (size) containing media (soil, sand, leaf manure and compost in proportion of (2:1:1:1).The pot was covered with polythene sheet to preserve moisture. These pots were firstly placed in conditions similar to in vitro (16 h photoperiod and at temperature 25 ± 2°C.

Refrences

Anonymous (2008). "Annual world statistics". (weblink: http://stat.fao.org). Access date: 09-01-2010.

Asghar. S., Ahmad, T., Hafiz, I.A., Yaseen, M. (2011). "In-vitro propagation of orchid (Dendrobium nobile) var.Emma white". Afr. J. Biotechnol. 10(16): 3097-3103.

Berg, A.J.W., Zhang, X.P., Rhodes, B.B. (1997). "Micropropagation of Citrullus lanatus (Thumb.) Matsum and Nakai (watermelon)". Biotech. in Agric. and Forestry. 39: 124-129.

Bornmant, C.H., (1983). "Possibilities and constraints in the regeneration of tree from cotyledonary needles of Picea abies in vitro". Plant. Physiol. 36: 57-60.

Chaturvedi, R., Bhatnagar, S.P. (2001). "High frequency shoot regeneration from cotyledon explants of watermelon cv. sugar baby". In vitro Cell. Dev. Biol. 37: 255-258.

Choi, P.S., Soh, W.Y., Kim, Y.S., Yoo, O.J., Liu, J.R. (1994). "Genetic transformation and plant regeneration of watermelon using Agrobacterium tumefaciens". Plant Cell Rep. 13: 344-348.

Compton, M.E. Gray, D.J. (1993). "Shoot organogenesis and plant regeneration from cotyledons of diploid, triploid and tetraploid watermelon". J. Am. Soc. Hort. Sci. 118: 151-157.

Compton, M.E., Gray, D.J., Gaba, V.P. (2004). "Use of tissue culture and biotechnology for the genetic improvement of watermelon". Plant Cell. Tiss. Org. Cult. 77: 231-243.

El-Zeiny, O.A.H. (2007). "The highest population of plantlets from somatic embryogenesis and economical evaluation of Cucumber (Cucumber sativus L.) in vitro." J. Appl. Sci. Res. 3(11): 14 60-1471.

Gambley, R.L., Dodd, W.A., (1990). "An in vitro technique for production of de novo of multiple shoots in cotyledon explants of cucumber (Cucumis sativus L.)". Plant Cell Tiss. Org. Cult. 20: 177-183.

George, E.F., Hall M.A. & DeKlerk, G.J. (2008). "Plant Propagation by tissue culture". 3rd Edition. Springer, 1: 1-504.

Graster, H. (1997). "The potential role of lycopene for human health". J. Am. Coll. Nutr. 16: 109-126.

Handley, L.W. & Chambliss, O.L. (1979). "In-vitro propagation of Cucumis sativus L". Sci. Hort. 14: 22-23.

Hasan, S.Z.U., Ahmad, T., Hafiz, I.A. & Hussain, A. (2010). "Direct plant regeneration from leaves of Prunus Rootstock GF-677 (Prunus amygdalus x P. persica)". Pak. J. Bot. 42(6): 3817-3830

Hoque, A., Islam, R. & Joarder, O.I. (1995). "In-vitro plantlets differentiation in kakrol (Mamordica dioica Roxb.)". Plant Tiss. Cult. 5: 119-124.

Jaskani, M.J., Kwon, S.W. & Kim, D.H. (2005). "Comparative study on vegetative reproduction and qualitative traits of seven diploid and tetraploid watermelon lines". Euphytica 145: 259-268.

Kihara, H. (1951). "Triploid watermelons". Proc. Amr. Soc. Hort. Sci. 58: 217-230.

Lucier, G. & Lin, H.B. (2001). "Factors affecting watermelon consumption in the United States". Vegetables and specialties: situation and outlook. VGS-287 (p. USDAERS pp. 23-29).

Marr, C.W. & Gast, K.L.B. (1991). "Reaction by consumers in a "farmers" market to price for seedless watermelon and ratings of eating quality". Hort. Technol. 1: 105-106.

Mroginski, L.A. & Kartha, K.K. (1981). "Regeneration of plants from callus tissue of the forage legume Stylosanthes guianensis" Plant Sci. Lett. 23: 245-251.

Niedz, R.P., Smith, S.S., Dunbar, K.B., Stephens, C.T. & Murakishi, H.H. (1989). "Factors influencing shoot regeneration from cotyledonary explants of Cucumis melo". Plant Cell. Tiss. Org. Cult.18:313-319.

Pirinc, V., Onay, A., Yildirim, H., Adiyaman, F., Isikaln, C. & Basaran, D. (2003). "Adventitious shoot organogenesis and plant regeneration from cotyledons of diploid diyarbakir watermelon (Citrullus lanatus cv. "surme")". Turk. J. Biol. 27: 101-105.

Rafique, R., Fatima, B., Mustaq, S., Iqbal, M.S., Rasheed, M., Ali, M. & Hasan, S.Z.U. (2012). "Effect of indole-3-butyric acid (IBA) on in vitro root induction in dendrobium orchid (Dendrobium sabin H.)". Afri. J. Biotech. 11(20):4673-4675.

Raharjo, S.H.T., Hernandez, M.O., Zhang, Y.Y., Punja, Z.K. (1996). "Transformation of pickling cucumber with chitinase-encoding genes using Agrobacterium tumefaciens". Plant Cell Rep. 15: 591-596.

Selvaraj, N., Vasudevan, A., Manickavasagam, M. & Ganapathi, A. (2006). "In-vitro organogenesis and plant formation in cucumber". Biol. Plant. 50 (1): 123-126.

Selvaraj, N., Vasudevan, A., Manickavasagam, M., Kasthurirengan, S., Ganapathi, A. (2007). "High frequency shoot regeneration from cotyledon explants of cucumber via organogenesis". Sci. Hortic. 112: 2-8.

Srivastava, D.R., Andrianov, V.M., Piruzian, E.S. (1989). "Tissue culture and plant regeneration of watermelon (Citrullus vulgaris Schrad. cv. Melitopolski)". Plant Cell Rep. 8: 300-302.

Sultana, R.S. & Bari, M.A. (2003). "Effect of different plant growth regulators on direct regeneration of watermelon (Citrullus lanatus Thumb)". Plant Tiss. Cult. 13(2): 173-177.

Sultana, R.S., BariMiah, M.A., Rahman, H., Rahman, M.M., Saddique, N.A. & Khatum, N. (2004). "In vitro rapid regeneration of plantlets of plantlets from leaf explant of water melon (Citrullus lanatus Thumb.)". Biotechnol. 3(2): 131-135.

Thomas, T.D., Sreejesh, K.R. (2004). "Callus induction and plant regeneration from cotyledonary explants of ash gourd (Benincasa hispida L.)". Sci. Hortic. 100: 359-367.

This academic article was published by The International Institute for Science, Technology and Education (IISTE). The IISTE is a pioneer in the Open Access Publishing service based in the U.S. and Europe. The aim of the institute is Accelerating Global Knowledge Sharing.

More information about the publisher can be found in the IISTE's homepage: http://www.iiste.org

CALL FOR PAPERS

The IISTE is currently hosting more than 30 peer-reviewed academic journals and collaborating with academic institutions around the world. There's no deadline for submission. **Prospective authors of IISTE journals can find the submission instruction on the following page:** http://www.iiste.org/Journals/

The IISTE editorial team promises to the review and publish all the qualified submissions in a **fast** manner. All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Printed version of the journals is also available upon request of readers and authors.

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar