Journal of Biology and Today's World
ISSN 2322-3308
Journal home page: http://journals.lexispublisher.com/jbtw/

Received: 06 November 2015 • Accepted: 18 December 2015

Research

doi:10.15412/J.JBTW.01040803

In vitro regeneration of watermelon seed segments

Maryam Ameri[1,2*], Mehrdad Lahouti[2], Abdolreza Bagheri[3], Ahmad Sharifi[4], Fatetemeh Keykha Akhar[4,5]

[1] Industrial microorganisms biotechnology, Iranian academic center for education, culture and research, branch of Mashhad, Mashhad, Iran

[2] Biology department, College of science, Ferdowsi university of Mashhad, Mashhad, Iran

[3] Biotechnology and plant breeding department, College of agriculture, Ferdowsi university of Mashhad, Mashhad, Iran

[4] Ornamental plant biotechnology, Iranian academic center for education, culture and research, branch of Mashhad, Mashhad, Iran

[5] Department of plant biotechnology, College of agriculture, Jahrom University, Jahrom, Iran

*correspondence should be addressed to Maryam Ameri, Industrial microorganisms biotechnology, Iranian academic center for education, culture and research, branch of Mashhad, Mashhad, Iran; Biology department, College of science, Ferdowsi university of Mashhad, Mashhad, Iran; Tell: +989124731496; Fax: +9851338810177; Email: ma.ameri88@gmail.com.

ABSTRACT

Various parts of a seed have different responses to same culture condition, because of dissimilar hormone concentration and enzyme activity. Indirect regeneration of watermelon seed was tested with different parts: hypocotyl, basal, central, distal and peripheral zone (parts 1, 2, 3, 4 and 5, respectively). The different parts were cultured in MS medium containing 3.5 mg/l BA and 0.5 mg/l IAA. Photoperiod (16h light/8h dark) or dark condition were used as light treatment. Every 2 weeks the callogenesis and regeneration percentages and number of regenerated leaves were assessed until 6 weeks. Best callogenesis and regeneration percent observed in dark and light condition, respectively. A regular pattern of regeneration find in seed segments. The rate of regeneration was faster for second and third part of seed in compare to fourth and fifth parts ($p<0.01$). The peripheral parts show low density of regenerated plantlets. Regenerated branches were rooted in MS medium containing 0.1 mg/l IBA, and obtained plantlets with appropriate root system, were cultivated in hydroponic and sand pots to acclimatize. The best acclimatization (90 %) were gained in hydroponic culture

Key words: Citrullus lanatus, Seed segments, Regeneration

1. INTRODUCTION

Watermelon (*Citrullus lanatus*) a member of Cucurbitaceae, contain lots of water and is a good source of vitamin C, A and trace elements like calcium, potassium, and iron. Fruit of watermelon contain fiber which decrease blood cholesterol (1).Watermelon varieties differ in size, form, fruit color, design and color, and the seed maturation time. Most of watermelon varieties are diploid (2n=22), and triploid varieties which are sweeter and seedless, are produced during last 50 years. Also, there is tetraploid varieties, used to produce triploid hybrids. Iran is the third producer of watermelon with 1.9 million tons per year. Viruses are one of the major problems in watermelon plantation, which make lots of damages in watermelon fields worldwide. Production of high quality fruits like seedless fruits, or stress tolerant varieties that are resistant to viruses seems an efficient solution to this problem. Tissue culture and genetic manipulation are appropriate methods for implementing these solutions (2). One of the main objectives of watermelon breeding programs, is development of resistant varieties using DNA recombination technology (3). In addition, somaclonal variation in tissue culture seems as a right tool to produce and reproduce tetraploid plants. A large number of tetraploids are produced in tissue culture, which increase the number of appropriate tetraploid parents for production of seedless varieties (4). Watermelon is produced by clonal micropropagation of shoot apex and node, regeneration of adventitious shoot from cotyledon fragments and somatic embryogenesis. Adventitious shoot regeneration is reported in a wide range of diploid and tetraploid cultivars (3-5). In order to in vitro regeneration of watermelon, different explants including shoot apex (4), immature embryo (6), cotyledons (7), hypocotyle (5) and leaves (1)

are reported. In all reports, cotyledons of *in vitro* grown seedlings were the best explants. In first reports, auxin and cytokinin had been use for regeneration induction in watermelon cotyledons (8, 9). According to some reports benzyladenin (BA) is sufficient for shooting and auxins such as 2,4-D (2,4-Dichlorophenoxyacetic acid), NAA (1-naphthaleneacetic acid) and IAA (Indole-3-acetic acid) induce callus induction and inhibit regeneration (4, 5). Other cytokinins like Kinetin, 2ip (Isopentenyl Adenin), Zeatin and TDZ (Thidiazuron) are in the lower ranks of the BA for induction of branching (10). This study associated with evaluating the regeneration potential of different parts of watermelon seeds, and finding the best explant and different lighting condition for subsequent work.

2. MATERIALS AND METHODS

Watermelon seeds (*Citrullus lanatus* L. cv. Crimson Sweet) were carried out as the source of explant for indirect regeneration from different parts of the cotyledon. After sterilization seeds were washed with sterile distilled water. Then culturing in plates containing small amount of sterile water and were incubated for 72 h in dark to germinate. After softening the seed coat, it is isolated, and the cotyledons were sectioned into different parts. As shown in Figure 1, the areas investigated included hypocotyl, embryo, central part, distal area and the seed margins (1, 2, 3, 4 and 5, respectively).

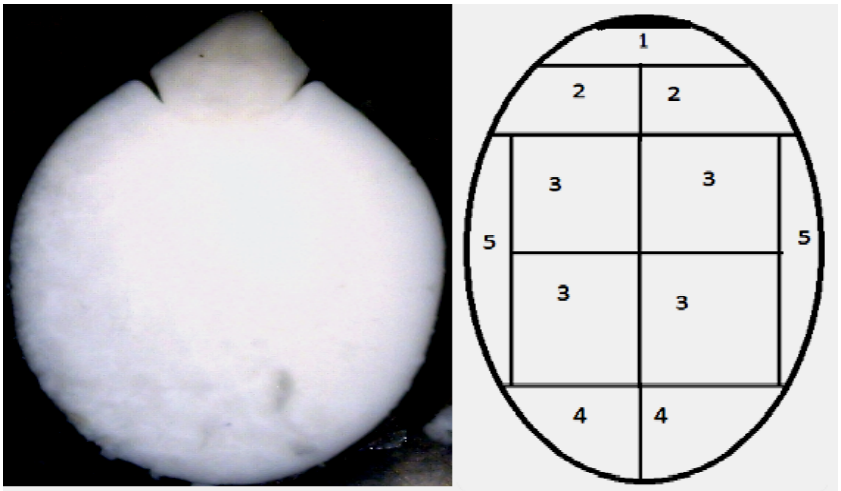

Figure 1. Different parts of seed. 1. hypocotyl, 2. embryo, 3. central part, 4. distal area and 5. seed margins

All parts of the seed were cultured in MS (Murashige and Skoog medium) media (11) with 3.5 mg/l BA in combination with 0.5 mg/l IAA. Samples were incubated in 25°c and 16:8 photoperiods, and subcultured after 3 weeks. Callogenesis and regeneration percentage, and the number of leaves and plantlets were recorded three times, once every 14 days. Regeneration percentage were calculated, based on the number of regenerated plantlets to total number of cultured explants. Each treatment consisted of four replicates, and each replicate was three instances. Evolved shoots were seprated and subcultured in vials containing 15 ml elongating medium (basal MS media). After 3 weeks, shoots longer than 1 cm were detached again, and were subcultured in rooting medium (MS medium with 0.1 mg/l IBA) for one month. In order to check acclimatization, seedlings with good root system were transferred to hydroponic culture, or a combination of sand and sterile perlit (1:1). Statistical methods used to analyze data in all experiments were a completely randomized factorial design.

3. RESULTS AND DISCUSSION

3.1. Investigation of regeneration potential from different parts of the seed

Based on the analysis of variance, light condition didn't have significant effect on callogenesis of different parts of the seeds (Figure 2).

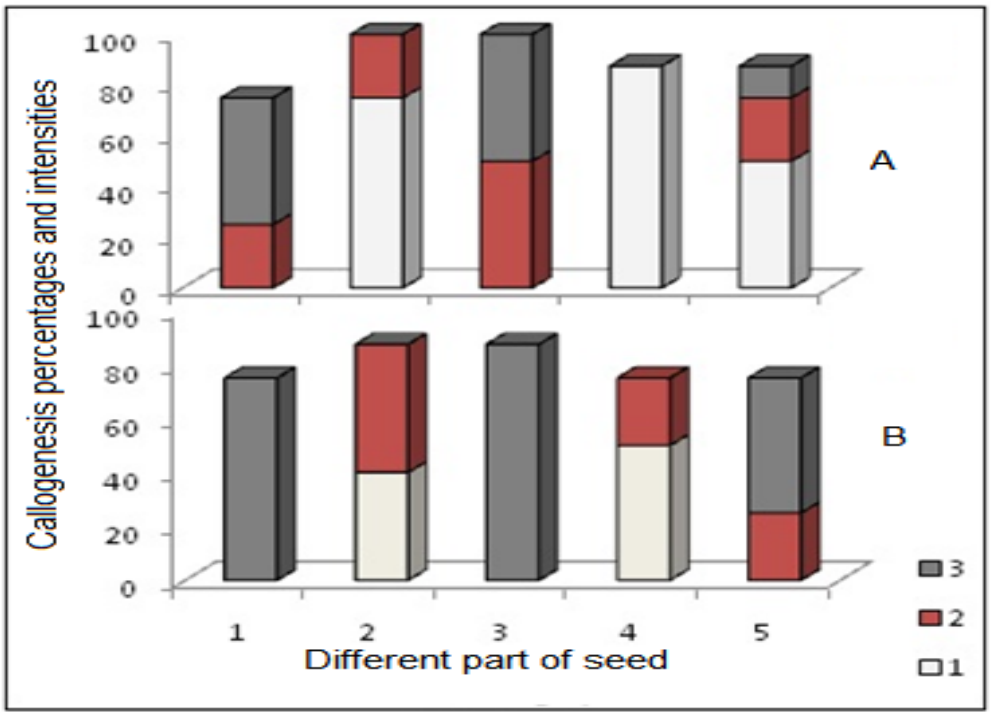

Figure 2. Callogenesis percentages and intensities of seed segments in A) light condition and B) dark condition (1. hypocotyl, 2. embryo, 3. central part, 4. distal area and 5. seed margins)

Intensity and type of induced calluses regenerated from different parts of seeds were clearly different. Regenerated calluses from part 1 were soft in both light and dark conditions, in a way that made subculture of these calluses difficult. Regenerated calluses from other parts, were so dense and fragile, that was so easy to move them. Different intensities of callogenesis were classified in three categories: week (number 1), average (number 2) and intensive (number3). Results showed that, the maximum intensity of callogenesis were in dark condition, and calluses from most of the explants, were in first and second categories. Maximum and minimum intensities were detected in parts 1, 3 and 2, 4, respectively (Figure 2). As we incubate seeds for 72 in dark, other reports indicated that seed germination in dark condition, increase the potential of organogenesis in cotyledons (12). Also, Compton (1999) reported that using dark pretreatment, increase shoot regeneration in cotyledonary explant. Such this result, is reported for other species, like tomato cotyledon and leaf explant (13), leaves explant of pear (14), potato (15), cotyledonary nodes of beans and peas (16), cotyledons, leaves and petioles of cucumber (17), dark treatment can influence the regeneration by protection and maintenance of endogenous growth regulators. Microscopic studies on etiolated tissues indicated that, they contain lots of paranchimic and undifferentiated cells. Since dedifferentiation is an important part of regeneration process, containing lots of undifferentiated cells improve the shoot regeneration (18). Etiolated tissues usually contain low polyphenolic compounds, small vascular tissue and thin cell wall (19). Transportation of growth regulators to the location of regeneration, will be facilitated by thin cell walls (18). Sometimes explants were kept in dark 1-2 months, and then calluses were transferred to light condition (20, 21). However, most reports in same line with our results, recommended the light and photoperiod of 16/8 as an important effective factor (10, 22-25). Results demonstrate that, different lightening conditions impress the amount of regeneration in different explants under various hormonal treatments. Although the best callogenesis were performed in dark condition, the best regeneration and organogenesis were recorded in light condition (Figure 2, Figure 3). It is significantly obvious, that light and dark condition were different ($p<0.01$) in regeneration responses (Figure 2). Light condition showed better regeneration response (64 % regenerated explants), in contrast to dark condition (25 % regenerated explants). Also different parts of seeds made significantly different regeneration responses ($p<0.01$). This response is reduced from part 2 to distal part. Maximum (92 %) and minimum (13 %) percent of regeneration were distinguished in part 2 and 5, respectively (Figure 3).

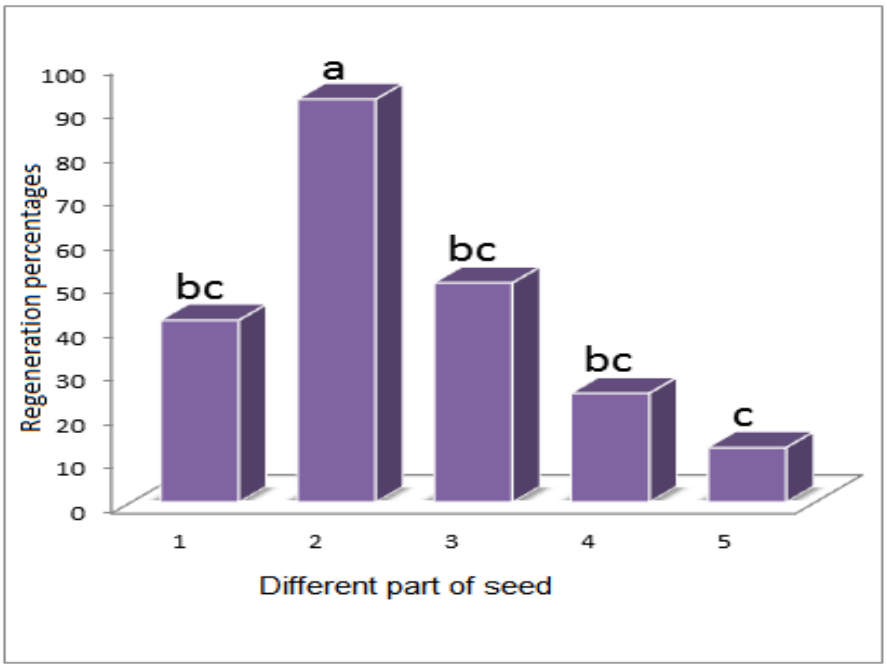

Figure 3. Regeneration percentages of seed segments (1. hypocotyl, 2. embryo, 3. central part, 4. distal area and 5. seed margins)

As we observed, other reports indicated that type of explant is an important factor in organogenesis, and cells with potential of producing lateral shoot are limited to a certain area (26-28). But there is only one report that declare distal part of watermelon cotyledon which is related to F1 hybrids (cvs. Sweet Gem and Gold Metal) had better regeneration (26). In a comprehensive study, Li et al. (24) used five different explants (cotyledon, distal and proximal area of cotyledon, nodal explant without proximal part and nodal explant without distal part). They noted the maximum induction of shoot regeneration (100 %) in nodal explants, and the maximum number of regenerated shoots in cotyledon and its proximal area. Ectopic regeneration of shoots occurred in petiole of cotyledon, which is near the embryo. They noticed that polarity appeared in tissue culture of watermelon is due to existence of meristems near the embryo and also the displacement of growth regulators in polar transports. In their experiment, distal area didn't show any regeneration, and only was swollen. Finally they introduce proximal area to agrobacterium infection (25, 29). Light condition made significant ($p<0.001$, 0.05) differences in number of regenerated leaves and plantlets (Figure 4).

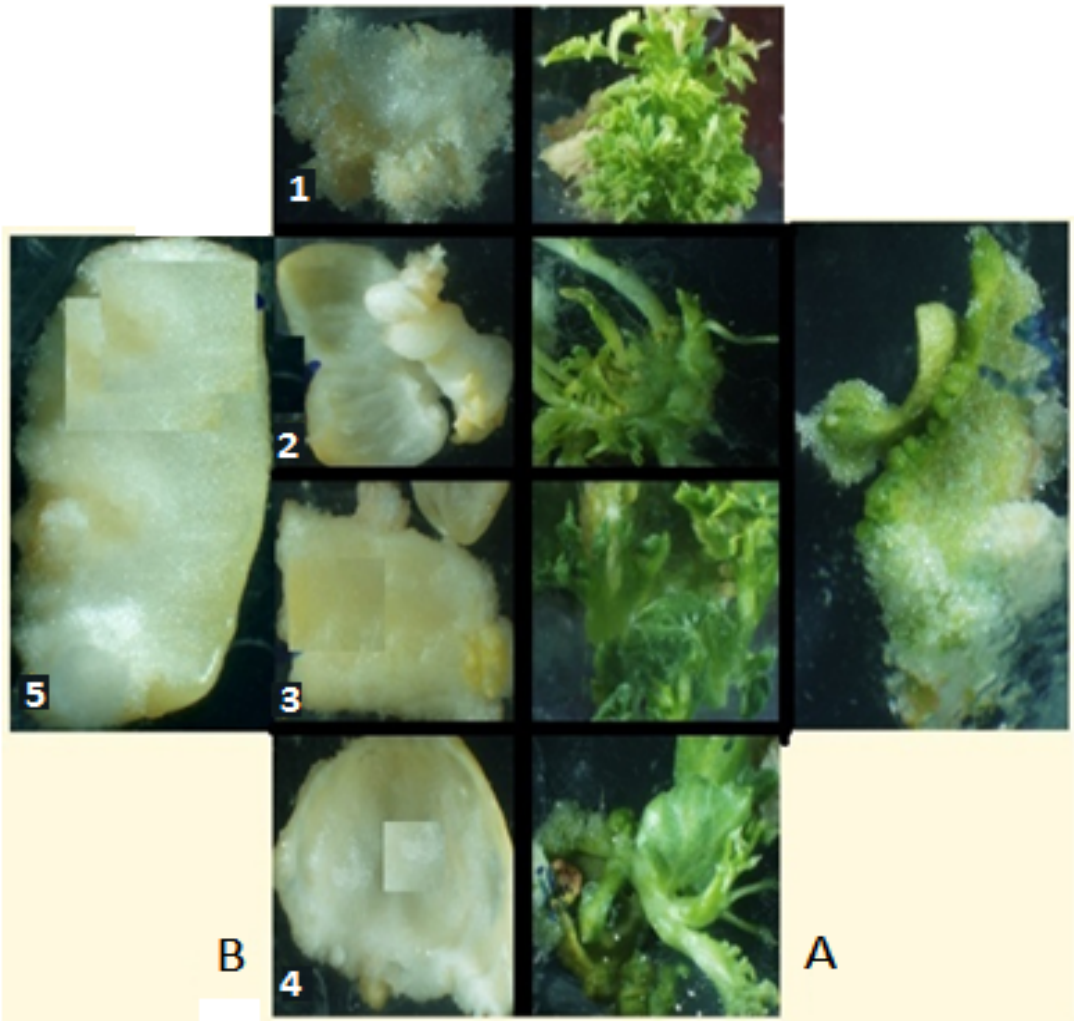

Figure 4. Regeneration percentages of seed segments in A) light condition and B) dark condition (1. hypocotyl, 2. embryo, 3. central part, 4. distal area and 5. seed margins)

In all samples light made better organogenesis ($p<0.01$). Maximum and minimum number of leaves and plantlets were recorded in explants of part 2 and 3, respectively. Results in higher and lower areas in contrast to part 2 and 3, were reduced significantly (Table 1).

Table 1. Different parts of seed; 1. Hypocotyl, 2. Embryo, 3. Central part, 4. Distal area and 5. Seed margins

Seed segments*	No. of planet regerated		No. of leaf regerated	
	Dark	Light	Dark	Light
1	0.26 cd	1.76 ab	0.38 d	4.13 bc
2	0.88 bc	2.88 a	1.88 cd	10.13 a
3	0.00 d	2.25 a	0.45 d	6.00 ab
4	0.00 d	0.13 cd	0.07 d	0.63 d
5	0.00 d	0.32 cd	0.00 d	0.63 d

Regenerated leaves from tip of the seed, were small and dense, while reaching bottom of the seed, greater number of leaves, were larger and less dense. In this slope of increase, results from part 3 were significantly different compared to part 2. Leaves and plantlets produced from part 2, and top sections of part 3, were directly regenerated. As indicated in this study and other reports, in cotyledon of watermelon, cells with potential of organogenesis are placed in proximal area (30), and because of this, most of the auxiliary buds developed in basal parts of explants (10, 27). Basal regions showed more frequent regeneration than apical region (7). Basal region showed a polarity that a lot of shoots regenerated near this region (20). Compton et al. (31) showed that basal parts, and vertically split to half cotyledons, had better regeneration results than four-part split cotyledons. Zhang et al. (32) noted that seeds without embryos, central basal region or region near immature cotyledons showed the most regeneration level. Also, this

result is demonstrated in mature seeds (30). Compton and Gray (33) and Compton (18) used basal region of diploid and tetraploid seeds. Using intact cotyledons especially in cultivars with low potential of regeneration, can lead to better regeneration results, in contrast to using half of the cotyledons (27).

3.2. Acclimatization

Plantlets with root and minimum length of 3 cm were moved to explain vitro condition. Hydroponically grown plants were more consistent (90%) than pot cultivation (65%) after 1month. At first plantlets had the average length of 4 cm and a simple root system, but one month after moving them to acclimatization condition, roots grown widely in plants with more than one m length, in hydropkick condition. Plants in greenhouse condition were not able to produce wide root and aerial system (Figure 5).

Figure 5. Acclimatization stages in A) sandy culture and B) hydroponic culture

It seems that hydroponic growth system were more successful in expansion of root system, compared with greenhouse culture system (Figure 5). During acclimatization in this system, root system suffer little damages (8). According to the fragility and damage of roots caused when exiting from vials and washing agar, also recording the rooting of plants without roots, in hydroponic culture with 0.1 mg/l IBA, it is suggested, that to decrease needed time, stages of rooting and acclimatization could be performed together, in hydroponic culture.

4. CONCLUSION

In this study, the effect of different part of watermelon seed besides hormonal and light treatment used to investigate callogenesis and regeneration in vitro. An obvious gradient pattern in proximal segment shows best regeneration and leave formation in compare to peripheral parts. Also, light condition induce more regeneration and less callogenesis in all parts. Hydroponic culture system increase growth rate and provide better access to nutrients. Also design of this system is flexible for large scale production.

Funding/Support

Not mentioned any funding/ support by authors.

ACKNOWLEDGMENT

This study was financially supported by ACECR, branch of Mashhad, Iran and Ferdowsi University of Mashhad.

AUTHORS CONTRIBUTION

This research paper was accomplished with the collaboration of all authors. Maryam Ameri performed the experiments, analyzed data and wrote the manuscript. Mehrdad Lahouti, Abdolreza Bagheri and Ahmad Sharifi designed the study and supervised the study. Fateme keykha Akhar helped us in performing and editing the manuscript.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

REFERENCES

1. Sultana R, Bari M, Rahman M, Rahman M, Siddique N, Khatun N. In vitro rapid regeneration of plantlets from leaf explant of water melon (Citrullus lanatus Thumb.). Biotechnology. 2004;3(2):131-5.
2. Compton ME, Gray D, Gaba VP. Use of tissue culture and biotechnology for the genetic improvement of watermelon. Plant cell, tissue and organ culture. 2004;77(3):231-43.
3. Dong J-Z, Jia S-R. High efficiency plant regeneration from cotyledons of watermelon (Citrullus vulgaris Schrad). Plant Cell Reports. 1991;9(10):559-62.
4. Compton ME, Gray D. Shoot organogenesis and plant regeneration from cotyledons of diploid, triploid, and tetraploid watermelon. Journal of the American Society for Horticultural Science. 1993;118(1):151-7.
5. Srivastava D, Andrianov V, Piruzian E. Tissue culture and plant regeneration of watermelon (Citrullus vulgaris Schrad. cv. Melitopolski). Plant Cell Reports. 1989;8(5):300-2.
6. Ahad A, Islam R, Hossain M, Khalekuzzaman M, Joarder O. Plant regeneration from immature and mature embryo axes of watermelon [in Bangladesh]. Plant Tissue Culture (Bangladesh). 1994.
7. Compton ME. Influence of seedling pretreatment and explant type on watermelon shoot organogenesis. HortScience. 1997;32(3):514-5.
8. Barnes LR. In vitro propagation of watermelon. Scientia Horticulturae. 1979;11(3):223-7.
9. Wilkins MC. Micropropagation of two triploid hybrids of Citrullus lanatus (Thunb.) Matsumura and Nakai1985.
10. Krug MGZ, Stipp LCL, Rodriguez APM, Mendes BMJ. In vitro organogenesis in watermelon cotyledons. Pesquisa Agropecuária Brasileira. 2005;40(9):861-5.
11. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia plantarum. 1962;15(3):473-97.
12. Bajaj Y. Biotechnology in agriculture and forestry: Springer-Verlag; 1997. 17 p.
13. Shahin EA. Isolation and culture of protoplasts: tomato. Cell culture and somatic cell genetics of plants. 2012;1:370-80.
14. Leblay C, Chevreau E, Raboin L. Adventitious shoot regeneration from in vitro leaves of several pear cultivars (Pyrus communis L.). Plant cell, tissue and organ culture. 1991;25(2):99-105.
15. Cheng J, Veilleux R. Genetic analysis of protoplast culturability in Solanum phureja. Plant Science. 1991;75(2):257-65.
16. Mohamed MF, Read PE, Coyne DP. Dark preconditioning, CPPU, and thidiazuron promote shoot organogenesis on seedling node explants of common and faba beans. Journal of the American Society for Horticultural Science. 1992;117(4):668-72.
17. Punja Z, Abbas N, Sarmento G, Tang F. Regeneration of Cucumis sativus var. sativus and C. sativus var. hardwickii, C. melo, and C. metuliferus from explants through somatic embryogenesis and organogenesis. Plant cell, tissue and organ culture. 1990;21(2):93-102.
18. Compton ME. Dark pretreatment improves adventitious shoot organogenesis from cotyledons of diploid watermelon. Plant cell, tissue and organ culture. 1999;58(3):185-8.
19. Hartmann HT, Kester DE. Plant propagation: principles and practices. Plant propagation: principles and practices. 1997(Edn 6).
20. Tabei Y, Yamanaka H, Kanno T. Adventitious shoot induction and plant regeneration from cotyledons of mature seed in watermelon. Citrullus lanatus.235-41.
21. Ibrahim IA, Nower AA, Badr-Elden AM, Elaziem TMA. High Efficiency Plant Regeneration and Transformation of Watermelon (Citrulus lanatus cv. Giza1). Res J Agric Biol Sci. 2009;5(5):689-97.
22. Okumuş V, Pirinç V, Onay A, Başaran D. In vitro propagation of Diyarbakır watermelons and comparison of direct-seeded and transplanted watermelon .Turk J Biol. 2011;35:601-10.
23. ONAY A, YILDIRIM H. Adventitious Shoot Organogenesis and Plant Regeneration from Cotyledons of Diploid Diyarbak› r Watermelon (Citrullus lanatus cv."Sürme"). Turk J Biol. 2003;27:101-5.
24. Li J, Li X, Qin Y, Tang Y, Wang L, Ma C, et al. Optimized system for plant regeneration of watermelon (Citrullus lanatus Thumb.). African Journal of Biotechnology. 2013;10(48):9760-5.
25. Li J, Tang Y, Qin Y, Li X, Li H. Agrobacterium-mediated transformation of watermelon (Citrullus lanatus). African Journal of Biotechnology. 2014;11(24):6450-6.
26. Choi PS, Soh WY, Kim YS, Yoo OJ, Liu JR. Genetic transformation and plant regeneration of watermelon using Agrobacterium tumefaciens. Plant Cell Reports. 1994;13(6):344-8.
27. Compton ME .Interaction between explant size and cultivar affects shoot organogenic competence of watermelon cotyledons. HortScience. 2000;35(4):749-50.
28. Ananthakrishnan G, Xia X, Elman C, Singer S, Paris H, Gal-On A, et al. Shoot production in squash (Cucurbita pepo) by in vitro organogenesis. Plant Cell Reports. 2003;21(8):739-46.
29. Suratman F, Huyop F, Wagiran A, Rahmat Z, Ghazali H, Parveez G. Cotyledon with hypocotyl segment as an explant for the production of transgenic Citrullus vulgaris schrad (watermelon) mediated by Agrobacterium tumefaciens. Biotechnology. 2010;9(2):106-18.
30. Compton ME, Gray D. Somatic embryogenesis and plant regeneration from immature cotyledons of watermelon. Plant Cell Reports. 1993;12(2):61-5.
31. Compton ME, Gray D, Elmstrom GW. A simple protocol for micropropagating diploid and tetraploid watermelon using shoot-tip explants. Plant cell, tissue and organ culture. 1993;33(2):211-7.
32. Zhang X, Rhodes B, Adelberg J. Shoot regeneration from immature cotyledons of watermelon. Cucurbit Genet Coop Rep. 1994;17:111-5.
33. Compton ME, Gray D. Adventitious shoot organogenesis and plant regeneration from cotyledons of tetraploid watermelon. HortScience. 1994;29(3):211-3.