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COMPARATIVE TISSUE CULTURE STUDIES OF TOMATO CULTIVARS ROMA, CHERRY-NARC AND NAGINA

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ABSTRACT

A comparative *in vitro* study was carried out for 3 tomato cultivars, Roma, Nagina and Cherry NARC. Two sources of explant were used; one was hypocotyl and another one was cotyledon leaves. MS media which was fortified with different concentration of phyto hormones was used to evaluate efficacy and the regeneration potential of the explant respectively. Among those three tomato varieties; tested for their callusing and regeneration potential, Roma responded as compared to Nagina and Cherry-NARC. Among all five different Medias tested in this regard MS media fortified with phyto hormones 2.5 mg/L BAP induced 76.6% callus from cotyledonary explants of Roma. MS media supplemented with IAA (0.2 mg/L) + BAP (2.0 mg/L) was found to be most efficient for shoot induction with an overall average of 27.78%. All the shoots were cultured on rooting media supplemented with MS media containing 0.2 mg/L of IAA.

Keywords: Indole-3-Acetic Acid, 6-Benzylaminopurine, Cotyledon, Hypocotyl, MS media

INTRODUCTION

Tomato (*Solanum lycoparsicum* Mill.) turns out to be one of the extensively grown vegetables across the world. It is an important food crop across the globe **[1]**. It is member of Solanaceae family. Farmers prefers to cultivate this crop as it has high yield potential as well as it is a short duration plant which makes it economically important crop for generating revenue in short pace of time. Tomatoes are rich in nutrients, mineral salts, ascorbic acid, lycopene, carotenes *etc*. **[2]**. Among 15 vegetables enlisted by the researcher, tomato has attained sixth position when compared to other vegetables and fruit regarding total annual production **[3]**. Traditional methods of breeding are laborious and time taking. It is troublesome to modify or improve the crops through conventional breeding techniques. Plant cell and tissue culture comes as a simple and effectual regeneration system to bring about desired outcome in short span of time and cost effective manner. It has been revealed in different studies that regeneration of different tomato cultivar requires optimization of specific tissue culture protocol by keeping different factors in consideration like plant variety, age of explant, source of explants, physical factors, type and composition of tissue culture media **[4-7]**. One of the biggest challenges for success of tissue culture protocol in organogenesis is genotype; different genotypes behave differently in the same media. This made genotype as one of the limiting factor for *in vitro* plant regeneration, thus it makes it mandatory to establish and optimize specific tissue culture protocol for different genotypes. Morphogenetic factor greatly impact the behavior of tomato plants during *in vitro* regeneration **[8]**. Selection of type and source of explant is also a vital factor for successful clonal propagation of tomato plant, different sources of explants such as cotyledonary leaves, hypocotyls **[9, 10]**, apical meristem **[9]**, root segments, nodes **[11]** are mostly used for this purpose. Some plant varieties are considered recalcitrant or stubborn or non-competent verities as these do not give positive response to *in vitro* growth and transformation. Phyto hormones carry a significant weightage in tissue culture success. Different combinations of plant hormones have been used in different studies. An efficient cytokinin and auxin combination can induce significant shoot induction in different plant cultivars and different sources of explants **[9, 12]**. Tissue culturing is categorized as an important technique in bringing biotechnological improvement such as production of virus resistant crops, regeneration of commercially important crops through clonal propagation and genetic transformation of crops like tomato **[13]**. Most of the commercialized tomato varieties do not yield at maximum which is linked to lack of suitable growing condition. One of the major issues with tomato production is hot climatic condition as most of the tomato cultivars are sensitive toward high temperature which in turn results in decreased tomato production in summer season **[14, 15]**.

MATERIAL AND METHOD

Acquisition of plant seeds

Seeds for three tomato cultivars Roma, Nagina and cherry NARC were acquired from HRI (Horticulture Research Institute), NARC (National Agriculture research center), Islamabad, Pakistan.

Culture media and growth conditions

MS media **[16]** incremented with 30 g/L sucrose and 7g/L agar was employed. The pH of the media was adjusted to 5.8 before autoclaving the culture media at 121 °C and 1.05 kg /cm^2 (15-20 psi) for 15 minutes. Different concentrations of phyto hormones BAP (6-Benzylaminopurine) and IAA (Indole-3-acetic acid) hormones were added in the media before autoclaving.

Preparation and germination of seeds

To break dormancy the seeds of *Solanum lycoparsicum* L, seeds of Roma, Nagina, and Cherry NARC were inundated in sterilized distilled water for 2 days at the temperature of 4°C. The sterilized seeds were inoculated on Basal MS media **[10]**. These inoculated seeds were kept in the dark for about one week until the germination initiation was observed. Then these were placed under sixteen hours of photo period, 25±2ºC temperature, with 50 µmolm-2s-1 fluorescence light and 60-70% relative level of humidity. Seedlings aged two to three weeks were deployed for in vitro studies.

Tissue culturing protocol

In vitro grown 2-3 weeks old seedling was used as explant source. + leaves and hypocotyl were secluded from the seedling and used as explant for callus induction. Hypocotyl of 0.5 cm and leaf disc of 3mm were inoculated in test tubes, glass jars and magenta boxes on five different Medias listed in **Table 1**. The effect of media, explant source and cultivar was analyzed on the basis of total number of explant producing callus, total number of callus producing shoots and number of shoots generating roots. All shoots attaining the height of approximately 1.5 cm were shifted to rooting media supplemented with 0.2 mg/L IAA. All the treatments were performed in three replicates with 30 explants in each treatment. The jars were incubated under sixteen hours of photoperiod, 25±2ºC temperature, with 50 µmolm-2s-1 fluorescence light. Sub culturing of explant was done every second week on the same media.

Table 1: Composition of culture media with different combination of Plant hormones

Media	Composition of Media	Phyto hormones
TCM1	MS media,30 g/L sucrose, 7.0 g/L agar	1.0 mg/L BAP+ 0.5 mg/L IAA
TCM2	MS media,30 g/L sucrose, 7.0 g/L agar	2.0 mg/L BAP+ 0.2 mg/L IAA
TCM3	MS media,30 g/L sucrose, 7.0 g/L agar	1.0 mg/L BAP
TCM4	MS media,30 g/L sucrose, 7.0 g/L agar	2.5 mg/L BAP
TCM5	MS media,30 g/L sucrose, 7.0 g/L agar	0.2 mg/L IAA

***TCM Tissue culture media**

RESULTS AND DISCUSSION

Factors like the smaller DNA genome as compared to other widely studied plants has made tomato classified as one of the best crop for genetic transformation studies **[17, 18]**. Plant tissue culture is a fundamental part of plant genetic engineering. Although there are many reports of successful *in vitro* tomato plant regeneration, however one of the limiting factors is that the *in vitro* response of tomato, as it varies for different cultivars **[19, 20]**. The i*n vitro* efficiency is dependent on composition of the media for tissue culture, especially plant phyto hormones secondly explant source is also a limiting factor for successful tomato transformation experiments (**Table 2**).

Callus Induction

Callus induction frequency was highest in cotyledonary leaves of Roma followed by Nagina and then Cherry NARC. The overall callus induction frequency of Roma was 55.16% which was significantly higher compared to 38.67% and 31.51% callusing frequency of Nagina and Cherry NARC respectively. These results show there is a direct correlation of callusing with explant source and type of cultivar, there are many studies focusing on the use of hypocotyl and cotyledon as an efficient explant source for *in vitro* studies of tomato. In the present study cotyledons as well as hypocotyls were used as an explant source. All three tomato cultivars were compared for their callusing potential on 5 different medias by using their cotyledons and hypocotyl as a source of explants. Callusing potential of Roma superseded other two cv Nagina and Cherry-NARC. Cotyledon explant of Roma demonstrated 76.67 % efficiency, while hypocotyl of Roma showed 62.22% callusing potential on MS Media 4 containing 2.5 mg/L of 6-Benzylaminopurine (BAP). Callusing frequency of 44.4.4 % from hypocotyl of Cherry- NARC and 41.11% was demonstrated by Nagina on media 4. Overall Roma demonstrated the best callusing potential. Cotyledons explants are more efficacious in callus induction as compared to hypocotyl our results show a similarity with the previous study of Sharma **[17]**, who reported cotyledon explant as the most suitable callusing explant source. Among the 5 different media used callus induction percentage was significantly high 44.44% with media 4 (2.5 mg/L BAP) followed by 48.87% with media 2(2.0 mg/L BAP +0.2 mg/L IAA). From cotyledonary explants Cherry-NARC showed 43.33% and Nagina showed 38.89% callusing potential onTCM2. The higher callusing rate on MS media fortified with 2.5mg/L of phyto hormone 6-Benzylaminopurine (BAP) alone or in combination of 2mg/L of the 6-Benzylaminopurine and 0.2 mg/L of phyto hormone Indole-3-acetic acid (IAA) goes in close agreement with previous reports of Gerszberg **[21]**; who reported best shooting response on MS media with 2 mg/L BA and 0.1 mg/L IAA for tomato cultivar 'Malinowy Warszawski' as well as 'Luban'. In our study, the highest callus induction frequency was observed for cultivar Roma and Cherry-NARC with media 4 (2.5 mg/L BAP) followed by media 2 (2.0 mg/L BAP +0.2 mg/L IAA), which is in agreement with the previous reports where higher cytokinin to auxin ratio has been shown to enhanced callus formation and shoot regeneration in tomato **[4]**. Similarly, other studies have also concluded that a higher concentration of cytokinin with relatively lower concentrations of auxins were responsible for a higher percentage of the callus formation **[22, 23] (Figure 1)**.

Shoot induction

There is a noteworthy outcome of source of explant on shooting rate; cotyledon explant supersedes hypocotyl in shoot formation. Among all the cultivar under observation Roma proved to be a best in shoot induction 13.55% compared to Nagina (4.64%) and Cherry-NARC (4.86%). Cotyledon explants of Roma showed highest shoot induction compared to Nagina and Cherry-NARC. So cultivar Roma supersedes other cultivars. Cotyledonary source of explant is more efficient compared to hypocotyl explant source. The interaction between cultivar, explant source and media has shown that TCM2 containing 2mg/L BAP+0.2 mg/L IAA was more efficient among other media sources with tomato variety Roma, when cotyledonary leafs were used as an explant source. Shooting frequency with TCM2 (17.24%) was highest among all other media. It was followed by TCM4 (2.5 mg/L BAP) with the shoot induction frequency of 16.40 %. Cherry NARC also showed good response (13.33%) on media 2 when hypocotyls were used as explant source. Overall cotyledon explants exhibited better shooting induction compared to hypocotyls, this result is in close proximity to many previous reports **[17, 24]**. Different sources of explant have been utilized in different studies. Some of them found hypocotyl superior over the cotyledons for the regeneration of shoots **[25-27]**, However the findings of Chaudhry, **[10]** are in close proximity to our result who found cotyledons more prone to shoot induction compared to other sources of explant for tomato cultivar Roma. **(Table 3 and Figure 3)**.

Rooting

Rooting is most important stage of *invitro* studies. It has been found that tomato can develop roots even in the absence of hormones from outside source **[28]**. This assumption is supported by the presence of adequate amount of endogenous auxin which makes rooting possible even in the absence of exogenous source of auxin **[25, 29]**. One study **[25]** reported root induction from nodal explants of tomato when they placed them vertically in the MS media without any phytohormone, which clearly supports the rooting possibility without adding auxin from outside source. Some studies revealed that addition of auxin in moderate amount helps in rapid and strong rooting of the shoots **[30]**. In the present study all shoots were shifted to the rooting media containing 0.2 mg/L of IAA. Healthy roots were induced within 2-4 weeks. Our results are in close agreement with the previous studies of **[31]** who reported rapid and 100% regeneration of healthy roots on MS media supplemented with 0.1-0.2 mg/L of IAA **[21, 32-34]** found that shoots cultured on MS media having 0.1 mg\L IAA produced profuse healthy roots while roots developed without exogenous IAA were long and fragile. Plants are naturally supplemented with phyto hormones for in vivo growth and development, however for in vitro plant regeneration sometimes exogenous supplementation of plant growth regulators is carried out to achieve optimum growth. Combination and concentration of cytokinin and auxin is directly related to genotype of tomato and it varies for different cultivars. Time period required for organogenesis has strong connection with the exposure time, combination and concentration of plant growth regulator used **[35-38]**. In vitro tomato regeneration can be carried out directly as well as through callusing. Cytokinin shows an crucial effect for induction of shoot, different studies revealed that if the media is supplemented with higher cytokinin level compared to auxin or if cytokinin is used alone in the culture media it will onset shoot regeneration from callus **[39-43]**.

Table 2: Callusing percentages of 3 tomato varieties on 5 different Medias from two explant sources

	Genotypes	TCM1	TCM2	TCM3	TCM4	TCM5	Mean
Hypo	Roma	24.44	33.33	36.67	62.22	24.7	36.27
	Nagina	27.78	30	30	41.11	0	25.78
	Cherry-NARC	2	20	20	44.44	11.11	19.51
	Mean	18.07	27.78	28.89	49.26	11.84	27.17
Coty	Roma	28.89	44.44	21.11	76.67	17.78	37.78
	Nagina	28.89	38.89	18.89	20	22.2	25.774
	Cherry-NARC	32.22	43.33	16.67	22.22	5.56	24
	Mean	29.96	42.18	18.77	39.63	15.26	29.16
		TCM1	TCM2	TCM3	TCM4	TCM5	Mean
	Roma	26.66	30.55	32.78	69.44	18.31	35.548
	Nagina	28.33	34.44	24.44	30.55	11.1	23.87
	Cherry-NARC	17.11	31.66	18.33	33.33	8.33	21.75
	mean for media	24	32.21	25.18	44.44	12.58	27.68

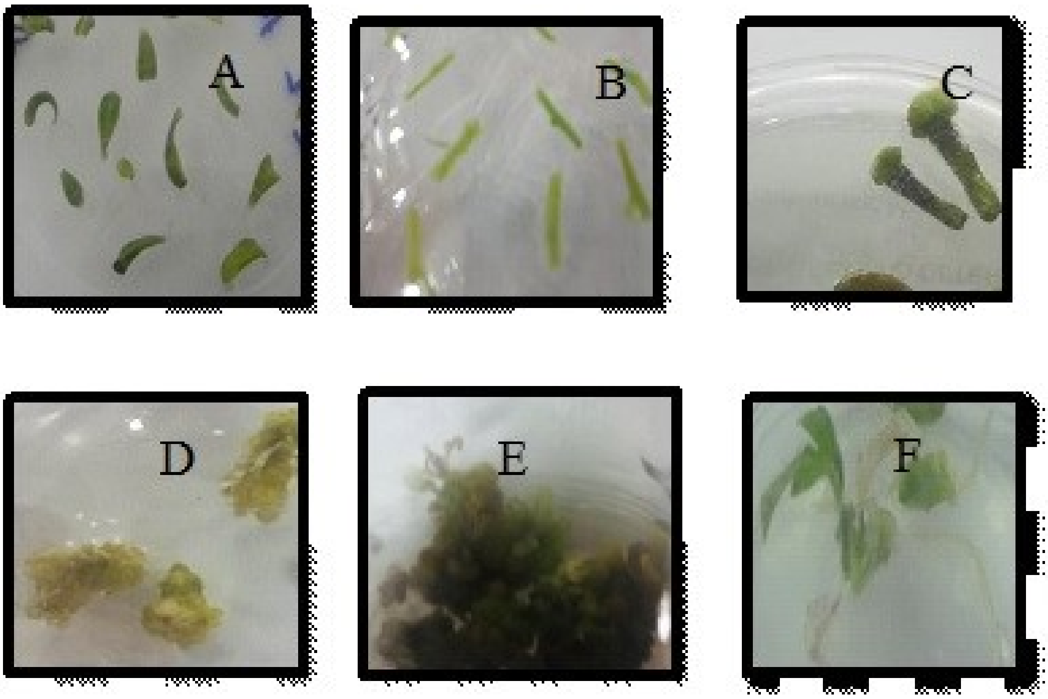

Figure 1: A; cotyledonary leaves explants B; hypocotyl explants C; callusing in hypocotyl derived explants D callusing from cotyledonary leaf derived explant E; shoot development from callus F; shoots developing roots

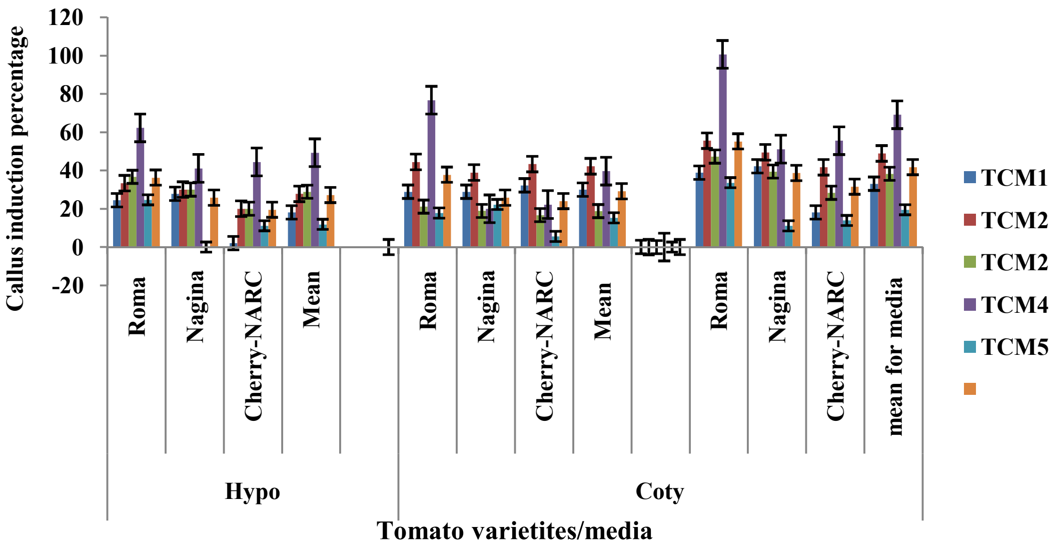

Figure 2: Comparisons of callus induction and role of 5 different Medias for 3 tomato varieties and 2 explant sources

Table 3: Shooting frequency of 3 tomato cultivars on 5 tissue culture Medias with 2 types of explants

	Genotypes	TCM1	TCM2	TCM3	TCM4	TCM5	Mean
Hypo	Roma	0	17.78	13.3	16.67	0	9.55
	Nagina	7.77	0	4.4	10	0	4.434
	Cherry-NARC	0	13.3	0	10	0	4.66
	Mean	4.81	10.43	5.9	12.29	0	6.686
Coty	Roma	18.88	20	16.66	6.66	5.55	13.55
	Nagina	3.3	11.11	8.8	0	0	4.642
	Cherry-NARC	2.2	10	0	7.7	4.44	4.868
``	Mean	7.97	13.63	5.15	8.23	3.48	7.692
		TCM1	TCM2	TCM3	TCM4	TCM5	
	Roma	9.44	27.78	21.63	20	2.775	16.32
	Nagina	9.42	5.555	8.8	10	0	6.75
	Cherry -NARC	1.1	18.3	0	13.85	2.22	7.09
	Mean for media	8.795	17.245	8.475	16.405	1.74	10.532

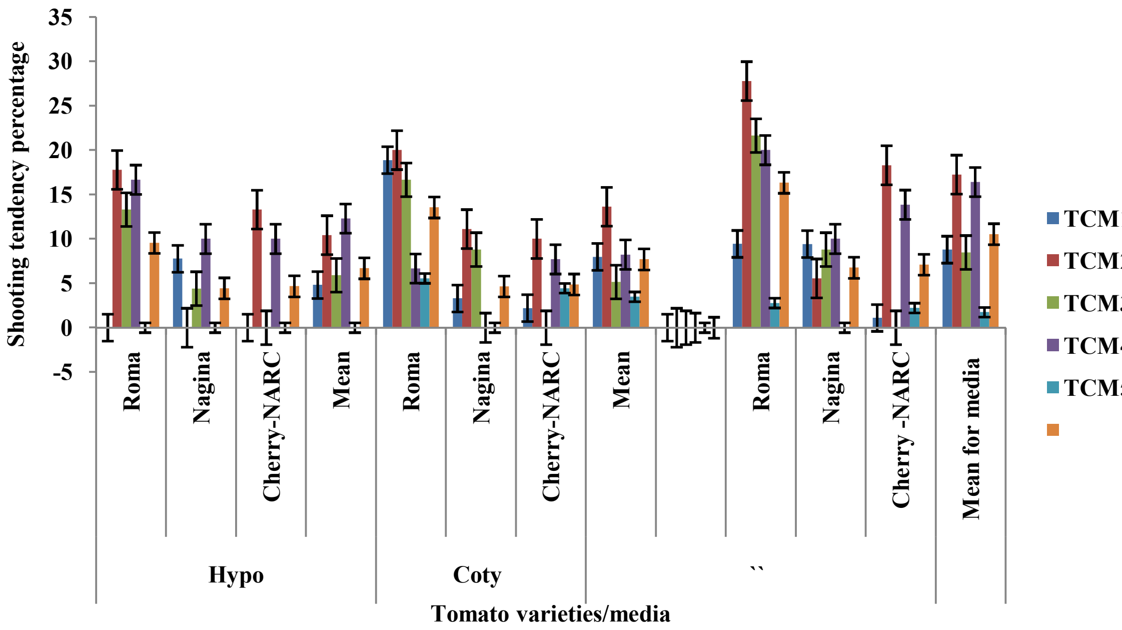

Figure 3: Comparative means representing shooting tendency of three tomato cultivars on five different Medias with two explant sources

CONCLUSION

Callusing was observed from both hypocotyl and cotyledonary leaves explants. However, cotyledonary source of explant dominated in callogenesis as well as shoot induction. Among all the tissue culture media used with different combinations of hormones, TCM4 with 2.5 mg/L BAP was found to be the best for callusing, for shooting media containing 2.0 mg/L BAP+ 0.2 mg/L IAA was most efficient. Among all three cultivars Roma proved to be the most efficient for shoot and callus induction.

REFERENCES

[1] Srinivas L, Kumar GS, Ganapathi T, Revathi C, Bapat V. Transient and stable expression of hepatitis B surface antigen in tomato (*Lycopersicon esculentum* L.). Plant Biotechnology Reports, (2008); 2(1): 1-6.

[2] Rukavtsova E, Zolova O, Buryanova NY, Borisova V, Bykov V, et al. Analysis of transgenic tobacco plants carrying the gene for the surface antigen of the Hepatitis B virus. Russian Journal of Genetics, (2003); 39(1): 41-45.

[3] Naz F, Haq IU, Asghar S, Shah AS, Rahman A. Studies on growth, yield and nutritional composition of different tomato cultivars in Battal valley of district Mansehra, Khyber Pakhtunkhwa, Pakistan. Sarhad J Agric, (2011); 27(4): 1-3.

[4] Park SH, Morris JL, Park JE, Hirschi KD, Smith RH. Efficient and genotype-independent Agrobacterium-mediated tomato transformation. Journal of plant physiology, (2003); 160(10): 1253-1257.

[5] Priya AM, Pandian SK, Manikandan R. The effect of different antibiotics on the elimination of Agrobacterium and high frequency Agrobacterium-mediated transformation of indica rice (*Oryza sativa* L.). Czech Journal of Genetics and Plant Breeding, (2012); 48(3): 120-130.

[6] Okkels F, Pedersen M. The toxicity to plant tissue and to Agrobacterium tumefaciens of some antibiotics. Bacterial and Bacteria-like Contaminants of Plant Tissue Cultures 225, (1987); 199-208.

[7] Shahriari Ahmadi F, Hashemi H, Hosseini B. Factor Influencing Regeneration and Genetic Transformation of Three Elite Cultivars of Tomato. Pakistan Journal of Biological Sciences, (2006); 9.

[8] Veluthambi K, Krishnan M, Gould JH, Smith RH, Gelvin SB. Opines stimulate induction of the vir genes of the *Agrobacterium tumefaciens* Ti plasmid. Journal of bacteriology, (1989); 171(7): 3696-3703.

[9] Ferdous M-E (2012) Establishment of in vitro regeneration and transformation protocol to develop salinity stress tolerant tomato (*Lycopersicon esculentum* Miller): BRAC Univeristy.

[10] Chaudhry Z, Rashid H. An improved Agrobacterium mediated transformation in tomato using hygromycin as a selective agent. African journal of biotechnology, (2010); 9(13): 1882-1891.

[11] Roy R, Purty RS, Agrawal V, Gupta SC. Transformation of tomato cultivar 'Pusa Ruby'with bspA gene from Populus tremula for drought tolerance. Plant cell, tissue and organ culture, (2006); 84(1): 56-68.

[12] Moghaieb RE, Saneoka H, Fujita K. Shoot regeneration from GUS-transformed tomato (*Lycopersicon esculentum*) hairy root. Cellular and Molecular Biology Letters, (2004); 9(3): 439-450.

[13] Ammon A, Reichart P, Pauli G, Petersen L. Hepatitis B and C among Berlin dental personnel: incidence, risk factors, and effectiveness of barrier prevention measures. Epidemiology & Infection, (2000); 125(2): 407-413.

[14] Yepes LM, Aldwinekle HS. Factors that effect leaf regeneration efficiency in apple, and effect of antibiotics in morphogenesis. Plant cell, tissue and organ culture, (1994); 37(3): 257-269.

[15] Takashina T, Suzuki T, Egashira H, Imanishi S. New molecular markers linked with the high shoot regeneration capacity of the wild tomato species Lycopersicon chilense. Japanese Journal of Breeding, (1998); 48(2): 109-113.

[16] Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia plantarum, (1962); 15(3): 473-497.

[17] Sharma MK, Solanke AU, Jani D, Singh Y, Sharma AK. A simple and efficient Agrobacterium-mediated procedure for transformation of tomato. Journal of biosciences, (2009); 34(3): 423-433.

[18] Rehman I, Aftab B, BILAL SARWAR M, Rashid B, Ali Q, et al. Gene expression in response to cotton leaf curl virus infection in *Gossypium hirsutum* under variable environmental conditions. Genetika (0534-0012), (2017); 49(3).

[19] Saidi M, Warade SD. Tomato breeding for resistance to Tomato spotted wilt virus (TSWV): an overview of conventional and molecular approaches. Czech Journal of Genetics and Plant Breeding, (2008); 44(3): 83-92.

[20] Riaz S, Malook S, Shah A, Sarfaraz M, Kazmi S, et al. Improvement of secondary metabolites for Cucurbita moschata through tissue culture techniques: An overview. Life Science Journal, (2015); 12(4): 94-101.

[21] Gerszberg A, Hnatuszko-Konka K, Kowalczyk T, Kononowicz AK. Efficient in vitro callus induction and plant regeneration protocol for different Polish tomato cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, (2016); 44(2): 452-458.

[22] Capote R, Fundora M, Pérez D. Effect of different factors on the in vitro plant regeneration from leaflets of five genotypes of tomato (*Lycopersicon esculentum* Mill.). Revista del Jardín Botánico Nacional, (2000); 21(1): 71-76.

[23] Hille J, Koornneef M, Ramanna M, Zabel P. Tomato: a crop species amenable to improvement by cellular and molecular methods. Euphytica, (1989); 42(1-2): 1-23.

[24] Düzyaman E, Tanrisever A, Guenver G. Comparative studies on regeneration of different tissues of tomato in vitro; 1993. pp. 235-242.

[25] Jehan S, Hassanein A. Hormonal requirements trigger different organogenic pathways on tomato nodal explants. American Journal of Plant Sciences, (2013); 4(11): 2118.

[26] Namitha KK, Negi PS. Morphogenetic Potential of Tomato (*Lycopersicon esculentum*) cv.'Arka Ahuti'to Plant Growth Regulators. Notulae Scientia Biologicae, (2013); 5(2): 220-225.

[27] Chaudhry Z, Abbas S, Yasmin A, Rashid H, Ahmed H, et al. Tissue culture studies in tomato (Lycopersicon esculentum) var. moneymaker. Pak J Bot, (2010); 42(1): 155-163.

[28] Bahurupe J, Patil S, Pawar B, Chimote V, Kale A. CALLUS induction and plantlet regeneration in tomato (*Solanum lycopersicum* L.). Journal of Cell & Tissue Research, (2013); 13(2).

[29] Bhatia P, Ashwath N, Senaratna T, Midmore D. Tissue culture studies of tomato (*Lycopersicon esculentum*). Plant cell, tissue and organ culture, (2004); 78(1): 1-21.

[30] Ashakiran K, Sivankalyani V, Jayanthi M, Govindasamy V, Girija S. Genotype specific shoots regeneration from different explants of tomato (*Solanum lycopersicum* L.) using TDZ. Asian Journal of Plant Science and Research, (2011); 1(2): 107-113.

[31] Otroshy M, Khalili Z, Ebrahimi MA, Nekoui MK, Moradi K. Effect of growth regulators and explant on plant regeneration of *Solanum lycopersicum* L. var. cerasiforme. Russian agricultural sciences, (2013); 39(3): 226-235.

[32] Hafeez M, Ahmad S, Mamoon-Ur-Rashid M, Ali A, Salman S, et al. AN overview of enhancing drought tolerance in cotton through manipulating stress resistance genes. Applied Ecology and Environmental Research, (2019); 17(3): 7003-7025.

[33] Qamar Z, Riaz S, Nasir IA, Ali Q, Husnain T. Transformation and evaluation of different transgenic lines for Glyphosate tolerance and cane borer resistance genes in sugarcane (*Saccharum officinarum* L.). Cytology and genetics, (2017); 51(5): 401-412.

[34] Bibi T, MUSTAFA HSB, Mahmood T, Hameed A, Ali Q. Multivariate analysis for adaptability and yield stability of rapeseed (*Brassica napus* L.)

strains in different agro-climatic zones. Genetika (0534-0012), (2018); 50(2).

[35] Puspito AN, Rao AQ, Hafeez MN, Iqbal MS, Bajwa KS, et al. Transformation and Evaluation of Cry1Ac+ Cry2A and GTGene in *Gossypium hirsutum* L. Frontiers in plant science, (2015); 6943.

[36] Aaliya K, Qamar Z, Nasir IA, Ali Q, Munim A. Transformation, evaluation of gtgene and multivariate genetic analysis for morpho-physiological and yield attributing traits in Zea mays. Genetika, (2016); 48(1): 423-443.

[37] Butt SJ, Varis S, Nasir IA, Sheraz S, Shahid A. Micro propagation in advanced vegetable production: A review. Advancements in Life Sciences, (2015); 2(2): 48-57.

[38] Riaz S, Shah AH, Ali Q. Study of Dichlorophenoxyacetic acid and 6-Benzylaminopurine effects on callus development in *Cucurbita moschata*. Molecular Plant Breeding, (2016); 7.

[39] Ali F, Ahsan M, Saeed NA, Ahmed M, Qurban A, et al. Establishment and optimization of callus-to-plant regeneration system using mature and immature embryos of maize (*Zea mays*). International Journal of Agriculture and Biology, (2014); 16(1).

[40] Majid MU, Awan MF, Fatima K, Tahir MS, Ali Q, et al. Phytophthora capsici on chilli pepper (*Capsicum annuum* L.) and its management through genetic and bio-control: a review. Zemdirbyste-Agriculture, (2016); 103(4).

[41] Majid MU, Awan MF, Fatima K, Tahir MS, Ali Q, et al. Genetic resources of chili pepper (*Capsicum annuum* L.) against Phytophthora capsici and their induction through various biotic and abiotic factors. Cytology and Genetics, (2017); 51(4): 296-304.

[42] Ali A, Iqbal M, Ali Q, Razzaq A, Nasir IA. Gene profiling for invertase activity: assessment of potato varieties for resistance towards cold induced sweetening. Advancements in Life Sciences, (2016); 3(2): 63-70.

[43] Awais M, Tariq M, Ali Q, Khan A, Ali A, *et al*. Isolation, characterization and association among phosphate solubilizing bacteria from sugarcane rhizosphere. Cytology and Genetics, (2019); 53(1): 86-95.