Enhanced *in-vitro* embryogenesis and multiple shoot regeneration of wheat local cvs (*Triticum aestivum* L.) with coconut water

Running Title: Enhanced wheat regeneration with coconut water

Asma Rafique, Amber Afroz*, Nadia Zeeshan

Department of Biochemistry and Biotechnology, University of Gujrat, Gujrat, Punjab, Pakistan

Abstract: Increasing human population and food demands necessitate the development of biotechnological strategies for improved crop production. Plant tissue culture is a crucial step for wheat genetic transformation. Callus induction and regeneration of 3 local wheat cultivars; Faisalabad 2008, Galaxy and Zincol monitored: with different concentrations of 2, 4-D, coconut water, and BAP. The mature seed embryo was an explant source. All the cultivars showed maximum callus induction at 3 mgL^{-1} of 2, 4-D; this was further enhanced by 20% coconut water. The highest callus induction was 84.6% for Faisalabad 2008, 74.6% for Galaxy, and 63.3% for Zincol. Regeneration frequency also varied significantly with coconut water. The highest regeneration was with 1.5 mgL^{-1} BAP, 10% coconut water for Faisalabad 2008 (81.6%), and the least in Zincol (68.3%). Faisalabad 2008 was found most responsive among three wheat cultivars for callus induction, proliferation, and regeneration. Coconut water was found efficient and vital for *in vitro* wheat regeneration. Chlorophyll mutants were also observed; collected to study the genetic variability produced due to media effect. Plants were transferred to *in-vivo* conditions and the number of plants obtained by each variety was recorded.

Keywords: *Triticum aestivum*, callus induction, regeneration, coconut water, 2, 4 D

Abbreviations: Naphthalene acetic acid (NAA); 2, 4-Dichlorophenoxy acetic acid (2, 4-D); 6-Benzyl aminopurine (BAP); Murashige and Skoog (MS); Coconut water (CW).

1. Introduction

Wheat (*Triticum aestivum* L) is a self-pollinated annual plant: the main component of the human diet all over [1]. One of the three major crops, it is vital in meeting the challenge of continuously increasing the food need of the growing population [2]. Wheat is the most widely grown crop, with more than 218 million ha, providing 20% of the daily protein and food calories [3], [4]. It is the second most important food crop in the developing world after rice because 80 million farmers depend on wheat. Wheat research aims to augment the existing yield along with fighting against stresses. Resistance genes; were introduced against different pathotypes and environmental factors. Traditional breeding methods are used in Pakistan to improve the production and quality of the wheat crop. However, the limited availability of gene pool and the long duration of these methods are the main limitations for improving wheat by conventional methods [5]. Biotic and abiotic factors lowering the wheat production made the basis of tissue culture led to the genetic modifications [6]. Genetic engineering is less time-consuming without a species barrier. Any gene can be introduced into the wheat genome by any organism for the desired phenotype.

Wheat, like many monocots, is recalcitrant to *in vitro* culture and its regeneration and transformation are highly genotypic dependent [7]. The fundamental step in the plant transformation procedure is to optimize the tissue culture for callus induction and its regeneration. There are three main factors on which the tissue culture of wheat depends,

culture medium and growth regulators [8], [9]. Explants sources used for wheat tissue culture included embryos (mature/immature), shoot bases, leaves, and root tips [10], [11]. *T. aestivum* immature embryos are widely reported explants for callus induction: but due to the seasonal availability, alternatives such as mature embryos and immature embryos can be used [2], [12]. Mature embryos, seeds, or tissues derived from them are reported: as an effective alternative to immature embryos for their availability and genetic stability [7], [9], [13], [14], [15]. Growth regulator concentration in the media is crucial in callus growth. Low cytokinin and high auxin concentration are favorable for wheat callus growth [16].

The regeneration reluctance through callus is a drawback for monocots' genetic improvement, including wheat. Explants source, media composition, and genotype effects *in vitro* plant regeneration [7], [14]. Other factors affecting plant regeneration from wheat mature embryo/seed are sterilization, pH, combination, and concentration of growth hormones [13]. Mature wheat embryos callus induction and regeneration with auxins like Naphthalene acetic acid (NAA) and 2, 4-Dichlorophenoxy acetic acid (2, 4-D); and cytokinins like 6-Benzylaminopurine (BAP) and Kinetin are reported [8], [13]. Coconut water (CW) had wide use in the plant tissue culture industry for its growth-promoting regulator properties. It had the cytokinin-type activity (Zeatin riboside, isopentenyl adenine, kinetin), along with a source of sugars, vitamins, minerals, and amino acids [17], [18], [19]. The use of coconut water to enhance wheat regeneration has not been reported before.

There is a need to optimize regeneration using wheat mature seed embryos of local wheat cultivars. Somatic embryogenesis of local wheat varieties Faisalabad 2008, Galaxy and Zincol with CW and minimal growth regulators had so far not been explored. The present study will be a milestone for wheat genetic engineering through mature embryo tissue culture.

2. Research Elaborations

2.1. Plant Material

Mature wheat seeds of three local wheat cultivars: Faisalabad 2008, Galaxy, and Zincol seeds from Gujrat Pakistan, were selected for the experiment. The selection was through a survey and consultation with the agriculture extension department of Gujrat.

2.2. Sterilization

Mature wheat seeds were surface sterilized with 40% sodium hypochlorite with the drop of Tween 20 for 20 min with vigorous shaking. It was followed by washing in DD water thrice 20 min each. The seeds were dried, mature embryo excision followed by callus induction medium inoculation.

2.3. Callus induction and regeneration

Murashige and Skoog (MS) medium salts and vitamins, was used for callus induction, proliferation, and regeneration [20]. 1.5, 2, 2.5, 3, 3.5, and 4 mgL^{-1} 2, 4- D was used for callus induction. MS medium supplemented with optimized 2, 4-D concentration for callus induction; is used for callus maintenance and proliferation. MS with 2, 4-D best combination for callus induction was used, with 0.5, 10, 15, and 20% CW. Sucrose (30g) as carbon source and agar (8 gL^{-1}) as a gelling agent, added to one liter MS medium. PH of the medium adjusted to 5.7 for all experiments.

One mature embryo/test tube was inoculated and placed in a growth chamber (300 µmol m^{-2} s^{-1}, 16 h light/8 h dark) at 25°C and 70% relative humidity) for 15 days. All varieties were monitored, for callus induction, at different 2, 4-D

concentrations with and without CW. Calli were transferred to a maintenance medium after 15D to optimize 2, 4-D concentration for two weeks. After 15 days on maintenance medium, calli were shifted, on 0.5, 1, 1.5, 2, and 2.5 mgL^{-1} of BAP. The optimized BAP concentration was utilized, in combination with 0, 5%, 10%, 15% and 20% CW. Regenerated plants were transferred to a rooting medium consisting of MS media supplemented with 0.5% IAA. Rooted plants were transferred to pots containing soil containing organic manure in a growth room covered with polythene bags to maintain humidity. After 20D, the plantlets were transferred to the earthen pots.

2.4. Statistical Analysis

Three replicates were used for a hundred mature seed embryos/replicate for callus induction and regeneration. Means and STDEV used; for error bars in MS Excel. One-way analysis of variance (ANOVA); was used to find the significant difference between the concentrations of growth regulators response in different wheat varieties. Different alphabets represent the significant difference of the different varieties within the same media composition.

3. Results and Discussion

3.1. Effect of 2, 4-D, and CW on callus induction

Wheat callus induction and *in vitro* regeneration are highly genotypic dependent [4]. Optimization of an efficient in vitro regeneration system is a prerequisite for the protocols currently used for the successful genetic transformation of wheat cultivars. The situation limits the biotechnological application for wheat genetic improvement. In the present study, in vitro regeneration of three Pakistani wheat cultivars was achieved using a mature seed embryo with different callus induction and regeneration medium. Different cultivars showed substantial differences in the ability of embryogenic callus induction, callus growth (fresh weight of callus), rooting, and regeneration. Regeneration variation is attributed; to genetic difference and the embryogenic response for a different combination of growth regulators [4], [7], [9]. So different callus induction and regeneration medium tried for the best combination of a given genotype. Cultivars exhibited differential embryogenesis and regeneration percentages on induction and regeneration medium.

Three wheat cultivars Faisalabad 2009, Galaxy, and Zincol, were examined for their ability to induce callus using different concentrations of 2, 4-D. A compact and nodular callus for all the cultivars was obtained, characteristic of the embryonic callus, after 14D post-inoculation. Comparatively high concentrations of 2, 4-D for callus induction proved to be very efficient, vital auxin in callus induction [7], [9]. Genetic makeup is a reason for the different callus induction responses in different genotypes [10]. The data show that the ability to induce callus in all genotypes differed significantly from one another under the same concentration of 2, 4-D [11]. The highest calli number in all varieties was observed, with 3 mg L^{-1} of 2, 4-D (Fig 1A). Callus induction efficiency in all genotypes was 78% for Faisalabad 2008, followed by 70% in Galaxy and Zincol (50%) (Fig. 1A). From 1.5-2.5 mgL^{-1} of 2, 4-D callus induction varied from 28-61%: becoming maximum at three mgL^{-1} (78%), followed by a reduction in callus induction again with increasing 2, 4 D concentration from 3.5-4 (48-68%). The nutrient medium and genotype have a considerable influence on callus formation [21]. The highest callus induction was observed in Faisalabad 2008, while Zincol showed the minimum callus induction. Callus growth was significantly affected by CW (Fig 1B). Callus growth augmented by increasing CW concentration, become maximum at 10% (Fig 2A; 2B; and 2C).

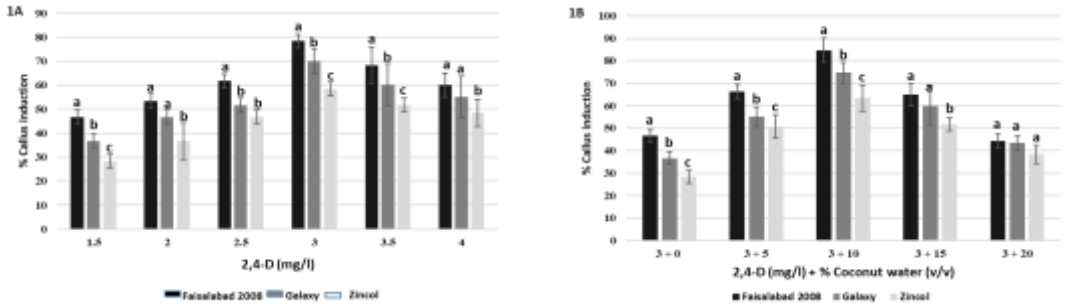

Fig 1. Callus induction percentage of *Triticum aestivum* cvs Faisalabad 2008, Galaxy, and Zincol with MS media supplemented with (1A) 1.5, 2, 2.5, 3, 3.5, and 4 mgL^{-1} of 2, 4-D. (1B) 3 mgL^{-1} 2,4-D with 5, 10, 15 and 20% coconut water.

Fig 2. Callus formation of *Triticum aestivum* cvs with MS media supplemented with 3 mgL^{-1} of 2, 4-D + 10 % coconut water. (2A) Faisalabad 2008 (2B) Galaxy (2C) Zincol.

The experiment was proceeded by using optimized 2, 4-D 3 mgL^{-1} with different concentrations of CW from 5-20%. The highest percentage callus induction was at three mgL^{-1} 2, 4-D, and 10% CW. That was 84.6% in Faisalabad 2008, followed by 74.6% for Galaxy and Zincol (63.3%). Further increasing CW concentration from 10-20% causes a reduction in callus. Enhanced callus induction leading to regeneration was observed, with 15% CW in tomato cvs [22]. Wheat mature embryos, maximum callus induction with 1.0 mgL^{-1} abscisic acid, 2, 4 D with 2.0 mgL^{-1} NAA is reported [14]. 2.0, and 0.2 mgL^{-1} 2, 4 D and NAA/4-chlorophenoxyacetic acid was found optimum for wheat callus; while regeneration is observed free of growth regulators from mature wheat embryos [7]. The highest callus induction with 1mgL-1 2, 4-D, 2 mg L^{-1} picloram, 200 mg L^{-1} casein hydrolysate using wheat mature/immature embryos was observed [9]. The combination of auxins was found superior for embryogenic callus induction; compared to a single auxin treatment previously. Mehmood et al. [23] reported 90% callus induction at 3mgL^{-1} of 2, 4-D in Inqalab-91 seeds. In this case, also best callus induction was found with single auxin alone or in combination with CW: which enhanced the callus.

3.2. Effect of 2, 4D, BAP, and CW on wheat regeneration

Regeneration is a vital step in tissue culture, as the response of different genotypes toward different growth regulators forms the basis for transformation. Embryogenic calli transferred to the regeneration media: with 0.5, 1, 1.5, 2, and 2.5 mgL^{-1} BAP 30D-Post-Inoculation. The results showed that regeneration frequency varies significantly with different BAP concentrations (Fig.3A, 4A, 4B, 4C). Faisalabad 2008 showed the highest regeneration (77.6 %) and proved to be better as compared to Galaxy (74.6 %) and Zincol (63.3 %) at 1.5 mgL^{-1} of BAP. An increase in BAP concentration from 0.5-1.5 mgL^{-1} augment regeneration for all wheat varieties, but a further increase in BAP from 1.5-2.5 mgL^{-1} led to reduced regeneration. 1.5 mgL^{-1} BAP in the regeneration medium is suitable for efficient regeneration in all types of wheat (Fig.

3A, 3B, 4). The highest regeneration of BAP at 1.5 mgL^{-1} was used with different concentration of CW (0, 5%, 10%, 15% and 20%) to check its effect on regeneration (Fig.3B; 5A, 5B, 5C, and 5D).

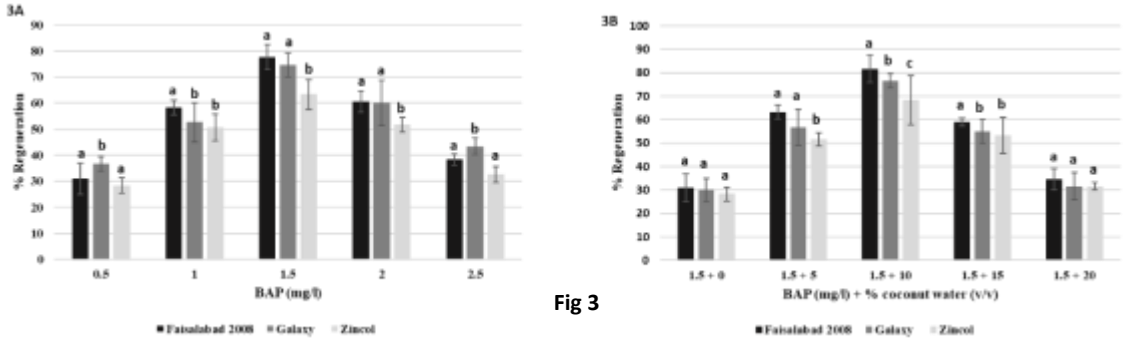

Fig 3

Fig 3. Regeneration percentage of *Triticum aestivum* cvs Faisalabad 2008, Galaxy, and Zincol with MS media supplemented with (3A) 0.5, 1, 1.5, 2, 2.5 mgL^{-1} of BAP (3B) 1.5 mgL^{-1} BAP with 5, 10, 15, and 20% coconut water.

The highest regeneration rate was for Faisalabad 2008 (81.6 %) and the least regeneration in Zincol (68.3 %), at 1.5 mg/l BAP with 10% CW. Regeneration decrease with a further increase in CW concentration which inhibits regeneration [22]. (Fig.3B; 4). Kumar *et al*. [14] regeneration was reported with BAP, thidiazuron, and NAA using mature wheat embryos. Regeneration from mature wheat seed callus (13.8 %) with 1.5 mg L^{-1} of IAA and 0.3 mgL^{-1} BAP is reported [8]. According to him, the callus induction and regeneration varied significantly with variety. So in this report, maximum regeneration was obtained with 10% CW and 1.5 mgL^{-1} BAP. Higher regeneration (59.33%) was observed in Chakwal-97 with 3mgL^{-1} BAP, while least regeneration was observed in Khyber (17.33%) at 4 mg L^{-1} BAP using seed source [23].

Fig 4

Fig 4. Regeneration of *Triticum aestivum* cvs with MS media supplemented with 1.5 mgL^{-1} BAP (4A) Faisalabad 2008 (4B) Galaxy (4C) Zincol.

Regeneration (65% & 52.38%) on MS medium containing 0.05 mg L^{-1} NAA using mature/immature embryos is reported [9]. Iqbal et al. [13] reported 4mgL^{-1} BAP for regeneration and maximum callus induction ranges from 4-6 mg L^{-1} 2, 4 D. In the given data, the combination of 10% CW and 1.5 mg L^{-1} BAP was found efficient for regeneration. *In vitro* wheat regeneration was found cultivar dependent, with Faisalabad 2008 signifying maximum regeneration. CW is a complex full of growth regulators like IAA, kinetin, and zeatin that minimize the cost of plant propagation [17], [18], [19]. CW was reported in olives and *Corylus avellana* L for embryogenic callus induction and micro-propagation [24], [25]. It's not been reported before in wheat, with the least number of growth regulators. Plants of wheat cvs obtained after rooting were shifted to soil and acclimatized (Fig 5F). Data is recorded for the plants obtained from all *Triticum* cvs (Table 1).

Fig 5

Fig 5. Regeneration of *Triticum aestivum* cvs with MS media supplemented with 1.5 mgL^{-1} of BAP and 10% coconut water 3W-Post inoculation (5A) Galaxy 2W-Post- inoculation (5B) Faisalabad 2008: 2W-Post inoculation (5C) Faisalabad 2008 3W-Post inoculation. Regeneration 3-W-post green spotted calli-inoculation (5D) Galaxy (5E) Zincol (5F) Faisalabad 2008**.**

Lack of chlorophyll, and results in photosynthesis impairment, resulted in abnormal plant development and premature death (Fig 5D, E). Albinism is frequently faced in tissue culture experiments. Nuclear mutation induces plastid ribosome deficiency; resulting in albinism [26], [27]. The molecular mechanism responsible for chlorophyll deficiency resulted in a mutant allele of the Alm gene for albino lemma and pericarp phenotype (line i: BwAlm) is limelight research now [26], [27]. A maximum number of chlorophyll mutants were observed in the variety Faisalabad 2008 and Zincol (90%) and minimum in Galaxy 66.67% (Table 1).

Table 1. Chlorophyll mutants obtained out of regenerated Triticum aestivum cvs obtained by mature embryos derived callus with different phytohormones.

Varieties	Total plantlets	Chlorophyll mutants	% Chlorophyll mutant
Faisalabad 2008	110	10	9.09
Galaxy	90	30	33.33
Zincol	62	08	12.9

The presence of chlorophyll deficient plantlets confirmed genetic variability [10], [28]. Phenotypic variability that occurred during *in-vitro* culture can revert to their parental type in field conditions.

4. Conclusion

The study showed the 2, 4-D efficiency in callus induction and proliferation at 3 mg L^{-1}) and the BAP in regeneration (1.5 L^{-1}). There was a significant difference between the three varieties, with superiority of the Faisalabad 2008 to Galaxy and Zincol. Along with this, CW significantly enhanced callus induction and regeneration. Faisalabad 2008 shows a maximum number of plantlets with minimum chlorophyll variation.

Declaration of Funding

The research funding is supported by National Research Program for Universities (NRPU 6506), Higher education Commission Islamabad, Pakistan at the Department of Biochemistry and Biotechnology, University of Gujrat, Gujrat Pakistan.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Statement

This article does not contain any studies with human participants or animals performed by the authors.

Consent for publication

The corresponding author had taken consent from all co-authors to the submission, and publication of their data in the Journal of Xi'an Shiyou University, Natural Science Edition.

Author Contribution

Asma Rafique (Experiment execution, Formal analysis, First draft preparation); Dr. Amber Afroz (Supervision, Experimental Design, Concept, Funding source); Dr. Nadia Zeeshan (Co-supervision, Concept, Paper Final editing).

References

[1] P. R. Shewry, "Wheat". J. Exp. Bot., vol. 60, no. 6, pp. 1537-1553, 2009.

[2] J. M. Zale, S. Agarwal, S. Loar, and C.M. Steber, "Evidence for stable transformation of wheat by a floral dip in Agrobacterium tumefaciens" Plant Cell Rep., vol. 28, no. 6, pp. 903-913, 2009.

[3] B. C. Curtis, "Wheat in the World". Available online: http://www.fao.org/3/y4011e/y4011e04.htm (accessed on 28 March 2019).

[4] P. Giraldo, E. Benavente, F. Manzano-Agugliaro, and E. Gimenez, "World-wide research trends on wheat and barley: A bibliometric comparative analysis". Agronomy, vol. 9, pp. 352, 2019. doi:10.3390/agronomy9070352.

[5] P. L. Bhalla, H.H. Ottenhof, and M.B. Singh, "Wheat transformation–an update of recent progress". Euphytica, vol. 149, no. 3, pp. 353-366, 2006.

[6] Z. Sramkova, E. Gregova, and E. Sturdik, "Chemical composition and nutritional quality of wheat grain". Acta Chim. Slov., vol. 2, no. 1, pp. 115-138, 2009.

[7] M. O. Adero, E.D. Syombua, L.K. Asande., N.O. Amugune, E.S. Mulanda, and G. Macharia, "Somatic embryogenesis and regeneration of Kenyan wheat (*Triticum aestivum* L.) genotypes from mature embryo explants". Afr. J. Biotechnol., vol. 18, no. 27, pp. 689-694.

[8] S. J. Baday, "*In vitro* study of the callus induction of two varieties of wheat seeds by plant growth regulators". Agric. J., vol. 13, no. 3, pp. 67-71, 2018.

[9] R. Ahmadpour, N. Zare, R. Asghari-Zakarta, and P. Sheikhzadeh, "Efficient i*n vitro* somatic embryogenesis and plant regeneration from mature and immature embryos of wheat (*Triticum aestivum* L.)". Braz. Arch. Biol. Technol., vol. 59, pp. e17160288, 2016. doi. 10.1590/1678-4324-201616028.

[10] S. Yasmin, I.A. Khan, A. Khatri, N. Seema, G. S. Nizamani, and M. A. Arain. "*In vitro* plant regeneration in bread wheat (*Triticum aestivum* L.)". *Pak. J. Bot.,* vol. 41, no. 6, pp. 2869-2876, 2009.

[11] M. M. Shah, Q. Khalid, U.W. Khan, S. A. H. Shah, S. H. Shah, A. Hassan, and A. Pervez, "Variation in genotypic responses and biochemical analysis of callus induction in cultivated wheat". Genet. Mol. Res., vol. 8, no. (3), pp. 783-793, 2009.

[12] S. Hayta, M. A. Smedley, M. Clarke., M. Forner, and W.A. Harwood. An efficient *Agrobacterium*-mediated transformation protocol for hexaploid and tetraploid wheat. Curr. Protoc., vol. 1, pp. e58, 2021. doi: 10.1002/cpz1.58.

[13] M. Iqbal, N.I. Raja, S. Asif, N. Ilyas, M. Hussain, F. Yasmeen, M. Ejaz, M.A. Sultan, S. Aslam, and H. Javed. *In-vitro* study of callogenesis and regeneration potential of elite wheat (*Triticum aestivum* L.) cultivars. Am. J. Plant Sci., vol. 7, pp. 2515-2526, 2016.

[14] A. Kumar, S. Sharma, N. L. Rai, A. Tomar, P. Kumar, R. S. Sanger, S. K. Singh, Vaishali, M. Kumar, and M. K. Yadav, "Callus induction and efficient plant regeneration in wheat (*Triticum aestivum* L.) through mature embryo culture". Int J Chem Stud, vol. 7, no. (4), pp. 1445-1450, 2019.

[15] Y. Yu, J. Wang, M. L. Zhu, and Z.M. Wei, "Optimization of mature embryo-based high frequency callus induction and plant regeneration from elite wheat cultivars grown in China". Plant Breed., vol. 127, no. 3, pp. 249-255, 2008.

[16] M. A. Mohammed, A. O. Alfalahi, A. S. Abed, and Z. N. Hashem, "Callus induction and plant regeneration from immature embryos of two wheat cultivars (*Triticum aestivum* L.)". Iraqi J. Biotech., vol. 18, no. 2,pp. 57-63, 2019.

[17] J. Arditti, "Micropropagation of Orchids", vol. II, 2nd ed. Blackwell Publishing, Oxford, UK, 2008.

[18] J. W. H. Yong, L. Ge, Y.F. Ng, and S.N. Tan, "The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water". Molecules, vol. 14, no. 12, pp. 5144-5164, 2009.

[19] M. L. Aguilar, F. Espadas, B. Maust, and L. Saenz, "Endogenous cytokinin content in coconut palms affected by lethal yellowing". J Plant Pathol., vol. 91, no. 1, pp. 141-146, 2009.

[20] T. Murashige, and F. Skoog, "A revised medium for rapid growth and bioassays with tobacco tissue cultures". Physiol. Plant., vol. 15, no. 3, pp. 473-497, 1962.

[21] R. Beyaz, "The effect of seed size on in vitro seed germination, seedling growth and tissue culture response of callus from mature embryos of wheat (*Triticum* sp.)". Fresenius Environ. Bull., vol. 28, no. 1, pp. 502-508, 2019.

[22] A. Afroz, Z. Chaudhry, U. Rashid, M.R. Khan, and G.M. Ali, "Enhanced regeneration in explants of tomato (*Lycopersicon esculentum* L.) with the treatment of coconut water" Afr. J. Biotechnol., vol. 9, no. 24, pp. 3634-3644, 2010.

[23] K. Mehmood, M. Arshad, G. M. Ali, and A. Razzaq, "Tissue culture responses of some wheat (*Triticum aestivum* L) cultivars grown in Pakistan". Pak. J. Bot., vol. 45, no. (SI), pp. 545-549, 2013.

[24] M. A. S. Prando, P. Chiavazza, A. Faggio, and C. Contessa, "Effect of coconut water and growth regulator supplements on *in vitro* propagation of *Corylus avellana* L". Sci. Hortic., vol. 171, pp. 91-94, 2014.

[25] R. A. V. de Souza, F.T. Braga, T.A. Setotaw, J.V. Neto, P.H. de Azevedo, V.H. de Azevedo, and G.M.D.A. "Cançado. Effect of coconut water on growth of olive embryos cultured *in vitro*". Cienc. Rural, vol. 43, no. 2, pp. 290-296, 2013.

[26] N. A. Shmakov, G.V. Vasiliev, N.V. Shatskaya, A.V. Doroshkov, E.I. Gordeeva, D.A. Afonnikov, and E.K. Khlestkina. Identification of nuclear genes controlling chlorophyll synthesis in barley by RNA-seq. BMC Plant Biol., vol. 16, pp. 245, 2016. https://doi.org/10.1186/s12870-016-0926-x

[27] J. J. Hanzel, J. P. Miller, M.A.B. Rinkman, and E. Fendos, "Genotype and media effects on callus formation and regeneration in barley". Crop Sci., vol. 25, pp. 27-31, 1985.

[28] O. Alikina, M. Chernobrovkina, S. Dolgov, and D. Miroshnichenko, "Tissue culture efficiency of wheat species with different genomic formulas". Crop Breed. Appl. Biotechnol., vol. 16, no. 4, 2016. https://doi.org/10.1590/1984-70332016v16n4a46.

Authors

First Author - Asma Rafique (MS), Department of Biochemistry and Biotechnology, University of Gujrat, Gujrat, Punjab, Pakistan, 16024379-007@uog.edu.pk

Second Author – Amber Afroz* (Ph.D.), Department of Biochemistry and Biotechnology, University of Gujrat, Gujrat, Punjab, Pakistan, dramber.afroz@uog.edu.pk.

Third Author – Nadia Zeeshan (Ph.D.), Department of Biochemistry and Biotechnology, University of Gujrat, Gujrat, Punjab, Pakistan, nadia.zeeshan@uog.edu.pk.

Corresponding Author-Dr. Amber Afroz (Ph.D.) (Assistant Professor), Email. dramber.afroz@uog.edu.pk, ambernics01@gmail.com, Phone: 0092-3359483609.