



Protein Profile of Tissue Culture of TRI2025 Tea Clone

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Abstract

Tea is well known as favourite healthy drink for almost all people over the world. Tea propagation using conventional and modern ways are now developing rapidly. However, information regarding the protein profile of tissue culture of tea plant has not been revealed yet. This study aimed to determine the difference of protein profile of tea's tissue culture using SDS-PAGE. This study was conducted using embryonic axes of TRI2025 tea clone cultured on MS media supplemented with 2,4-D for inducing somatic embryogenesis and globular-like structure (GLS) regeneration, and MS media supplemented with BAP for inducing shoot via organogenesis. The results revealed that proteins in the size of 37.69; 54.89; 60.77; 71.35; 87.34; and 92.99 KDa might be involved at somatic embryogenesis, and about 38.69 KDa, 69.27 KDa, and 55.76 KDa respectively for GLS, initiation of shoot, and initiation of GLS derived leaf. Predicted key protein for leaf initiation both directly or through GLS was about 31-33 KDa, while for callusing were about 27.56 and 52.73 KDa, and constitutive protein was about 22.75 KDa. This study provides the first report of protein profile of tea's tissue culture. The information obtained can be beneficial as a marker for explant for somatic embryogenesis, GLS, or organogenesis pathway and as a scientific information for further biotechnology development.

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INTRODUCTION

Tea (*Camellia sinensis* L.) is one of the most favourite drink in the world because of its secondary metabolite contents that useful to human health (Li *et al.*, 2015). Propagation systems of tea can be achieved by traditional or modern one. Formely, seed or stem cutting was the main technique to propagate, and then somenew techniques had arisen, such as grafting and micropogation. Micropropagation technique or known as “tissue culture” technique; can be achieved through somatic embryogenesis and organogenesis. Tissue culture uses somatic cells and utilizes totipotency in obtaining fully plants. Both of these technologies are now beginning to be developed as an alternative methods of tea plants (Kaviani 2013; Gonbad *et al.*, 2014).

Protein profile analysis in tissue culture is commonly used to determine protein condition at spesific time and location, so that the information about certain protein(s) involved at certain developmental stage can be known better and in detail. This information about protein profile can be useful for plant biotechnology. Protein profile analysis has been conducted in many tissue culture-derived plants (Huang *et al.*, 2012; Wang *et al.*, 2012), however, there has been no report about protein profile of tissue culture of tea until now. In this study, protein profile analysis was conducted using embryonic axis of TRI2025 tea clone, for inducing somatic embryogenesis, GLS, and organogenesis. Protein profile of tea from tissue culture can be an important information for further tea’s tissue culture development such as in genetic transformation development or in the process of tissue culture itself. Thus, the results of this study were expected to provide some information that can assist the better future of tea’s tissue culture development.

METHODS

Materials and Tissue Culture Conditions

Removed growth points of embryonic axes of TRI2025 tea clone were used as explants. Explants then were cultured on MS media supplemented with 2 mg.L⁻¹ 2,4-D for inducing somatic embryogenesis and GLS regeneration. Explants also were cultured in ½ MS media supplemented with 2 mg.L⁻¹ BAP to induce common leaves initiation through organogenesis pathway. Explants were incubated in light, with temperatures around 23°C. For protein profile analysis, samples were collected at callus stage, globular stage of somatic embryogenesis, GLS, common leaves

initiation, and initiation stage of GLS-derived leaves stage.

Protein Extraction and Protein Profile Analysis

Protein profile analysis was started by extracting soluble protein using phosphate buffer saline solution at pH 7, the protease inhibitor was added, and crushion was done. Afterward, centrifugation was done on 9000 rpm and after sedimentation, the supernatant was taken. Soluble protein concentrations were determined using the Bio-rad method. The determination of molecular weight (MW) of protein was done by Sodium Dodecyl Sulphate Poly Acrylamid Gel Electrophoresis (SDS-PAGE) method. Protein samples each with a final volume of 40µL were injected into gel well. Electrophoresis was performed at 100 volt for 2.5 hours. The next steps were staining with Coomassie brilliant blue 0.1%, color removal, and then kept in glacial acetic acid 10%. Molecular weight of protein (KDa) were determined by extrapolation (Khunsook *et al.*, 2003). In order to detect the spesific proteins that may play a role in different stage of tissue culture development, the obtaining protein was compared with protein profile at sampling stages using Image J (<https://imagej.nih.gov/ij/>).

RESULTS AND DISCUSSION

Callusing was the first phenomenon occurred on explants cultured on MS media supplemented with 2,4-D, but it was not occurred on explants cultured on MS media supplemented with BAP. This callus was in former incision of shoot apical meristem (SAM) and root apical meristem (RAM) that appeared at about 7 days after culture. The callus was a crumb in yellowish-white in color (Figure 1). This condition might be an effect of 2,4-D application since its role for inducing callogenesis, such as in *Piper betle* Var Nigra (Junairiah *et al.*, 2018), *Coriandrum sativum* L. (Ali *et al.*, 2017), and sugarcane (Tahir *et al.*, 2011).

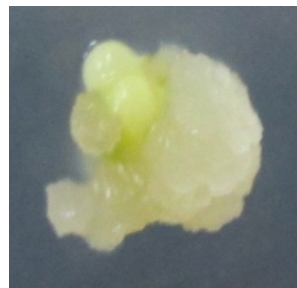


Figure 1. Morphology of tea callus

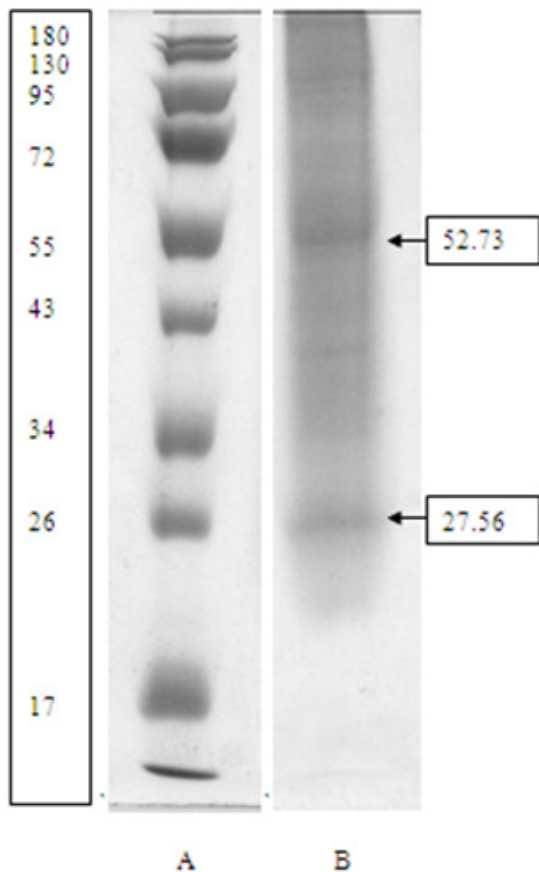


Figure 2. Protein profile analysis using SDS-PAGE. A: Marker; B: Callusing stage.

Callusing stage in this study was identified by appearance of specific protein of about 52.73 KDa and 27.56 KDa (Figure 2) from soluble protein. A protein of about 52.73 KDa was predicted to be similar with protein of about 52 KDa in callus of *Solanum nigrum* that was treated with 4 mg.L⁻¹AgNPs (Ewais *et al.*, 2015). A protein of about 52 KDa was also reported in UV-elicited callus of *Eurycoma longifolia* and then was predicted as GPP synthase enzyme (Parikrama & Esyanti, 2014). Other specific protein at callusing stage was approximately 27.56 KDa which was in line with study by Balen *et al.* (2002) that reported a protein of about 27 KDa in rooty tumour mutant in cactus. This tumour might be correlated with crown gall; another name of callus. Ewais *et al.* (2015) also reported the appearance of 27 KDa protein in 2 mg.L⁻¹AgNPs-treated callus of *Solanum nigrum*. These reports suggested and reinforced the possibility of a specific 52.73 and 27.56 KDa protein involved at callusing stage.

There were several similarities and differences among proteins derived from somatic embryo, GLS, initiation stage of commonleaves, and initiation stage of GLS derived leaves (Figure 3).

Soluble protein of about 22.75 KDa was appeared at all induced organs and this protein might be involved in house-keeping function in plant. It was allegedly included in small heat shock proteins (sHSPs), with MW ranged from 15-42 KDa (Zhao *et al.*, 2018) and correlated with heat-stress condition (Song *et al.*, 2017). In transgenic tea, it has been confirmed that 17.2 KDa of sHSP has a capability to respond to thermo-stress condition (Wang *et al.*, 2017). However, a study by Rani *et al.* (2009) reported that protein of about 22 KDa was found in somatic and zygotic embryo stage of peanut. Mishra *et al.* (2012) also reported that in late stage of somatic embryo of chickpea, there was a protein ranged from 22-24 KDa identified as Late Embryogenesis Abundant (LEA) protein, or Legumin β that has a molecular weight of about 20 KDa.

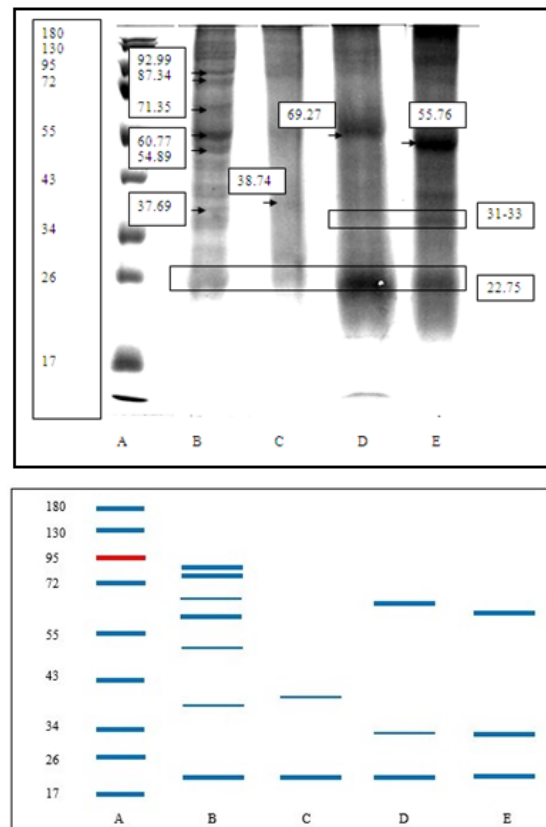


Figure 3. Protein profile using SDS-PAGE (up) and its representative (down). A: Marker; B: somatic embryo (globular stage); C: GLS; D: initiation stage of common leaves; E: initiation stage of GLS derived leaves.

There was 37.69 KDa protein found at somatic embryo sample. In line with that condition, 36-38.1 KDa protein was also found in *Capsicum chinense* somatic embryo with pI value ranged

from 8.8-9.8. That protein band was also found in embryo of *P. taeda* (Brownfield *et al.*, 2007) that was later predicted as storage protein. Mishra *et al.* (2012) reported that storage protein in chickpea that was between 34-37 KDa was associated with vicilin minor subunit that appeared at different stages of somatic embryo. However, study by Tonietto *et al.* (2012) reported that a protein of about 37 KDa in somatic embryo of coffee was later identified as Glyceraldehyde 3-phosphate dehydrogenase; a kind protein correlated with glycolysis, and also to be involved in plant defence reaction against reactive oxygen species (ROS) such as hydrogen peroxide (Hancock *et al.*, 2005). Therefore, this could be highlighted that in early somatic embryo stage required proteins to control cell damage from ROS and has some storage proteins.

Later, a protein of about 54.89 KDa was also found and expected to be associated with Vicilin major subunit that has MW of 48-50 KDa (Mishra *et al.*, 2012). In coffee somatic embryo, it was also reported a protein that has MW of about 54.1 KDa and later was identified as 11s storage globulin (Tonietto *et al.*, 2012). A protein of about 60.77 KDa was also only found at somatic embryo and expected to be associated with Chitinase and this protein was also reported by Fraterova *et al.* (2013) at somatic embryo cell line of pinus. Rani *et al.* (2005) also reported a protein of about 65 KDa in thick band of immature zygotic embryo that subsequently used for explants of somatic embryo. These two previous reports confirmed the result of this study that found a protein of about 60 KDa at somatic embryo sample.

A protein of about 71.35 KDa was found at somatic embryo stage, and it was in line with Mishra *et al.* (2012) that reported a kind of storage protein included in convicilin; in range between 68-72 KDa. Tonietto *et al.* (2012) was also reported that protein with MW of about 70.7 KDa was identified as member of Hsp70 protein. Hsp70 protein is correlated with stress condition and is needed for plant defence (Usman *et al.*, 2017). These previously studies strengthened our study's result that somatic embryo stage required proteins to defence and store the energy for further stages.

A protein of about 87.34 KDa was also found at somatic embryo stage, and it was predicted to be correlated with 82 KDa protein found at further stage of carrot's somatic embryo (Chung & Pedersen, 1998) or was predicted to be correlated with 85 KDa protein from somatic embryo of *Picea abies* (Mo *et al.*, 1996). Furthermore, Misra *et al.* (2012) reported that there was a kind

of storage protein identified as lipoxygenase at mature seed of chickpea with MW of about 96.4 KDa. Related to this study, there was also found a protein of about 92.99 KDa at somatic embryo. Another possibility of about 90 KDa soluble protein is the HSP90 protein that involved to somatic embryogenesis of *Pinus massoniana* L. (Zhen *et al.*, 2012) dan *Carica papaya* L. (de Moura Vale *et al.*, 2014). These previous studies supported our study's results that at somatic embryo stage really require proteins for storage and plant defence.

The only predicted protein appeared on GLS sample was about 38.74 KDa and was predicted a Ricin B lectin domain; it might be similar with putative r40c1 protein found in crown barley (Vitamas *et al.*, 2015). This protein might be related to stress condition especially at drought in rice (Ke *et al.*, (2009) and to be correlated with stress-response protein named Thioredoxin-like protein CDSP32 in purple young shoot during leaf development of field-land of tea (Zhou *et al.*, 2017). It was also predicted to be related to defence condition, such as reported by Xu *et al.*, (2013) regarding its appearance in rice that was exposed by rice black streaked dwarf virus (RBSDV) infection. Related to this result, this 38.76 KDa protein was predicted to be related to stress condition, considering the incision process at growth points of explants which gave it stress condition.

The main soluble protein found at initiation stage of common leaves was protein that have MW of about 69.27 KDa. This protein was correlated with 67 KDa protein found on leaf of *Arabidopsis thaliana* that was identified as Cytokinin-binding protein; a protein that plays important for leaf development (Selinvakina *et al.*, 2004). Other possibility of about 69.27 KDa obtained protein are Dihydrolipoamide dehydrogenase precursor or NADP-dependent glyceraldehyde-3-phosphate dehydrogenase that involved in expanding leaves of tea from field-land (Li *et al.*, 2015). It was also predicted to be related to Piruvate decarboxylase family protein or TCP-1/cpn60 chaperonin family protein, such as reported by Zhou *et al.* (2017) regarding its appearance in purple young shoot during leaf development in the field.

There were significant differences between initiation stage of common leaves and initiation stage of leaves through GLS pathway regeneration. One of them was a protein of about 55.76 KDa that thickly appeared in initiation leaves through GLS compared to initiation stage of common leaves. This protein might be correlated with Rubisco activity that commonly found on plant leaf (Kim *et al.*, 2017; Pottier *et al.*, 2018). Ma *et al.* (2009) reported that both of young and ma-

ture leaves contained a protein of about 52 KDa estimated as Chloroplast-encoded protein; large subunit of Rubisco protein. Other type possibility of 55.76 KDa is dehydrin, as reported Carjuzaa *et al.* (2008) from quinoa. Dehydrins or hydrophilins are protein correlated with dehydration condition or cold tolerance and belong to group II of LEA protein in plants (Liu *et al.*, 2017). Liu *et al.* (2015) reported that transformation using this gene could increase the cold tolerance of *Solanum habrochaites*, and also in *Arabidopsis thaliana* (Xie *et al.*, 2012). Another possibility of about 55.76 KDa obtained protein is Chloroplast phosphoribulokinase, as reported by Zhou *et al.*, (2014) in tea leaf from field-land subjected to drought, and correlated to Bark storage protein A, as reported by Zhou *et al.* (2017). Therefore, this could be concluded that at initiation of leaves through GLS had more proteins related to dehydration and photosynthetic-related protein(s).

The other band protein found both in initiation of common leaves and GLS derived leaves was about 31-33 Kda, predicted as Photosystem 2 (PS2) protein. This protein is important for light photosynthesis (Caffarri *et al.*, 2014). Nedunchezian *et al.* (2000) reported that there was slow inactivation of PS2 protein in 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) -treated wheat leaves at dark condition during senescens, but Mazraei (2016) reported that there was no significant effects of PS2 on dessication. Another possibility of about 31-33 KDa obtained protein is stress-response protein named Glutathione s-transferase, as reported by Zhou *et al.* (2017) in purple young shoot during leaf development of field-land of tea. These previous studies strengthen our study's result that in initiation stage of leaves both through organogenesis and GLS needed photosynthetic-related protein and plant defence protein.

One main different point between somatic embryo and GLS is the leaf-derived structure. In somatic embryo, leaf derived from development of cotyledone, but in GLS regeneration, leaf was derived from GLS. The GLS has difference in structure with globular stage of somatic embryo, that GLS is not fully in sphere structure as somatic embryo has. GLS is other pathway for tissue culture propagation of tea beside organogenesis and somatic embryo; or can be called by "transition regeneration" between them. This regeneration was first reported by Seran *et al.* (2006); called as small succulent leaves (SSL) but from unremoved embryo axis's growth points. This structure was also reported later in *Camellia nitidissima* (Lu *et al.*, 2013) and then called as nodu-

lar embryonic structures (NES).

Initiation stage of common leaves was different with GLS-derived leaves. In common leaves initiation, there was no GLS appearance at former incision of SAM, just common leaves initiation derived from leaf primordium. This result, once again, proved that different addition of plant growth regulator (PGRs) made different effect on same clone and same kind of explants. Although leaf initiation was primary controlled by auxin (Kalve *et al.*, 2014), this study also proved that inside of that explants might be contained sufficient quantity of endogen auxin that can influence the succesful of leaf initiation, although in media was supplemented with cytokinin; BAP. Also, it was proved that auxin is actually the main PGR(s) for promoting leaves initiation, and in this case, although it was preceded by GLS formation, auxin addition in media can induce leaves initiation, through that GLS structure.

This is the first report of protein profile of tissue culture derived from tea plant. Once again, this protein profil analysis just conducted using SDS-PAGE that made the obtained protein bands could not to be determined exactly, therefore, for further analysis, it will be more powerful analysis by using advanced technology, such as LC-MS spectroscopy. But, this results of this research provide scientific information about protein profile related to the somatic embryogenesis, GLS, and organogenesis using simple technique. Furthermore, for application benefits, such as key protein-formulated spray could be created from this scientific information.

CONCLUSION

Somatic embryogenesis and GLS can be induced by culturing the explants on MS media supplemented with 2,4-D. Initiation of common leaves can be induced by culturing the explants on ½ MS media supplemented with BAP. Protein profile analysis using SDS-PAGE revealed the predicted key protein for somatic embryogenesis were about 37.69; 54.89; 60.77; 71.35; 87.34; and 92.99 KDa, and the predicted key protein for GLS, initiation stage of common leaves, and initiation stage of GLS derived leaves were about 38.69 KDa, 69.27 KDa, and 55.76 KDa respectively.

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REFERENCES

- Ali, M., Mujib, A., Tonk, D., & Zafar, N. (2017). Plant regeneration through somatic embryogenesis and genome size analysis of *Coriandrum sativum* L. *Protoplasma*, 254, 343-352.
- Balen, B., Milo, J., & Rasol, M. K. (2002). Glycoprotein patterns related to morphogenesis in *Mammillaria agracillis* Pfeiff. *Tissue Culture, Food Technology, and Biotechnology*, 40(4), 275-280.
- Bertamini, M., Nedunchezian, N., & Borghi, B. (2001). Effect of iron deficiency induced changes on photosynthetic pigments, Ribulose-1,5-Bisphosphate Carboxylase, and photosystem activities in field grown grapevine (*Vitis Vinifera* L. cv. Pinot Noir) leaves. *Photosynthetic*, 39(1), 59-65.
- Brownfield, D. L., Todd, C. D., Stone, S. L., Deyholos, M. K., & Gifford, D. J. (2007). Patterns of storage protein and triacylglycerol accumulation during loblolly pine somatic embryo maturation. *Plant Cell Tissue Organ Culture*, 88(2), 217-223
- Caffarri, S., Tibiletti, T., Jennings, R.C., & Santabarbara, S. (2014). A comparison between plant photosystem I and photosystem II architecture and functioning. *Current Protein and Peptide Science*, 15(4), 296-331.
- Carjuzaa, P., Castellion, M., Distefano, A. J., del Vas, M., & Maldonado, S. (2008). Detection and subcellular localization of dehydrin-like proteins in quinoa (*Chenopodium quinoa* Willd.) embryos. *Protoplasma*, 233(1-2), 149-156.
- Chung, W. J., & Pedersen, H. (1998). Precipitated proteins improves the production of carrot somatic embryos. *Biotechnology Techniques*, 12(5), 417-420.
- de Moura Vale, E. D., Heringer, A. S., Barroso, T., da Silva Ferreira, A. T., da Costa, M. N., Perales, J. E. A., Santa-Catarina, C., & Silveira, V. (2014). Comparative proteomic analysis of somatic embryo maturation in *Carica papaya* L. *Proteome Science*, 12, 37-53.
- Ewais, E. A., Desouky, S.A., & Elshazly, E. H. (2015). Evaluation of Callus Responses of *Solanum nigrum* L. exposed to biologically synthesized silver nanoparticles. *Nanoscience and Nanotechnology*, 5(3), 45-56.
- Fraterova, L., Salaj, T., Matusikova, I., & Salaj, J. (2013). The role of chitinases and glucanases in somatic embryogenesis of black pine and hybrid firs. *Central European Journal of Biology*, 8(12), 1172-1182.
- Gonbad, R. A., Siniah, U. R., Azis, M. A., & Mohamad, R. (2014). Influence of Cytokinins in Combination with GA3 on Shoot Multiplication and Elongation of Tea Clone Iran 100 (*Camellia sinensis* (L.) O. Kuntze). *The Scientific World Journal*, doi. 10.1155/2014/943054.
- Hancock, J. T., Henson, D., Nyirenda, M., Desikan, R., Harrison, J., Lewis, M., Hughes, J., & Neill, S. J. (2005). Proteomic identification of glyceraldehyde 3-phosphate dehydrogenase as an inhibitory target of hydrogen peroxide in Arabidopsis. *Plant Physiology & Biochemistry*, 43(9), 828-835.
- Huang, H., Moller, M., & Song, S. Q. (2012). Proteomics of desiccation tolerance during development and germination of maize embryos. *Journal of Proteomics*, 75(4), 1247-1262.
- Junairiah, J., Purnomo, P., Utami, E. S. W., Ni'matuzahroh, N., & Sulistyorini, L. (2018). Callus induction of *Piper betle* Var Nigra using 2,4-dichlorofenoxyacetic acid and 6-benzil aminopurin. *Biosaintifika: Journal of Biology & Biology Education*, 10(3), 588-596.
- Kalve, S., Vos, D. D., & Beemster, G. T. S. (2014). Leaf development: a cellular perspective. *Frontiers in Plant Science*, 5, 362-387.
- Kaviani, B. (2013). Somatic embryogenesis and plant regeneration from embryonic axes and cotyledons explants of tea (*Camellia sinensis* L.). *Journal of Ornamental Plants*, 3(1), 33-38.
- Ke, Y., Han, G., He, H., & Li, J. (2009). Differential regulation of proteins and phosphoproteins in rice under drought stress. *Biochemical & Biophysical Research Communications*, 379(1), 133-138.
- Khunsook, S., Bary, S. B., R.M.G. Susan, R. M. G., & Jack, A. A. (2003). Purification and characterization of plasma membrane-associated human sperm α -L-fucosidase. *Biology of Reproduction*, 709-716.
- Kim, Y. J., Lee, H. M., Wang, Y., Wu, J., Kim, S. G., Kang, K. Y., Park, K. H., Kim, Y. C., Choi, I. S., Agrawal, G. K., Rakwal, R., & Kim, S. T. (2013). Depletion of abundant plant RuBisCO protein using the protamine sulfate precipitation method. *Proteomics*, 13(14), 2176-2179.
- Li, C. F., Zhu, Y., Yu, Y., Zhao, Q. Y., Wang, S. J., Wang, X. C., Yao, M. Z., Luo, D., Li, X., Chen, L., & Yang, Y. J. (2015) Global transcriptome and gene regulation network for secondary metabolite biosynthesis of tea plant (*Camellia sinensis*). *BMC Genomics*, 16, 560-580.
- Li, Q., Li, J., Liu, S., Huang, J., Lin, H., Wang, K., Cheng, X., Liu, Z. (2015) A comparative proteomic analysis of the buds and the young expanding leaves of the tea plant (*Camellia sinensis* L.), *International Journal of Molecular Science*, 16, 14007-14038.
- Liu, H., Yu, C., Li, H., Ouyang, B., Wang, T., & Zhang, J. (2015). Overexpression of ShDHN,

- a dehydrin gene from *Solanum habrochaites* enhances tolerance to multiple abiotic stresses in tomato. *Plant Science*, 231, 198–211.
- Liu, Y., Song, Q., Li, D., Yang, X., & Li, D. (2017). Multifunctional roles of plant dehydrins in response to environmental stresses. *Frontiers in Plant Science*, 8, 1018.
- Lu, J., Chen, R., Zhang, M., da Silva, J. A. T., & Ma, G. (2013). Plant regeneration via somatic embryogenesis and shoot organogenesis from immature cotyledons of *Camellia nitidissima* Chi. *Journal of Plant Physiology*, 170, 1202-1211.
- Ma, Z., Cooper, C., Kim, H. J., & Buckner, D. J. (2009). A Study of rubisco through western blotting and tissue printing techniques. *CBE—Life Sciences Education*, 8(2), 140–146.
- Mazraei, M. (2016). Influence of drought stress on lipids, root growth, PS2 activity and rubisco in crop plants. *Journal of Scientific and Engineering Research*, 3(4), 457-460.
- Mishra, S., Sanyal, I., & Amla, D. V. (2012). Changes in protein pattern during different developmental stages of somatic embryos in chickpea. *Biologia Plantarum*, 56(4), 613-619.
- Mo, L. H., Egertsdotter, U., & von Arnold, S. (1996). Secretion of specific extracellular protein by somatic embryos of *Picea abies* is dependent on embryo morphology. *Annals of Botany*, 77, 143-152.
- Nedunchezian, N., Muthuchelian, K., Bertamini, M. (2000). Photosystem 2 efficiency and thylakoid protein pattern in DCMU-treated wheat seedlings during senescence. *Photosynthetica*, 38(4), 607-614.
- Parikrama, R., & Esyanti, R. R. (2014). Effect of UV Elicitation on Callus Growth, Alkaloid and Terpenoid Contents in *Eurycoma longifolia* Jack. *International Journal of Advances in Chemical Engineering & Biological Sciences*, 1(1), 12-15.
- Rani, A. R., Reddy, V. D., Prakash Babu, P., & Padmaja, G. (2005). Changes in protein profiles associated with somatic embryogenesis in peanut. *Biologia Plantarum*, 49(3), 347-354.
- Selivankina, S. Y., Karavaiko, N. N., Maslova, G. G., Zubkova, N. K., Prokoptseva, O. S., Smith, A. R., Hall, M. A., & Kulaeva, O. N. (2004). Cytokinin-binding protein from *Arabidopsis thaliana* leaves participating in transcription regulation. *Plant Growth Regulation*, 43(1), 15–26.
- Seran, H. T., Hirimbregama, K., & Gunasekare, M. T. K. (2006). Direct somatic embryogenesis from explants obtained from in vitro germinated embryonic axes of *Camellia sinensis* (L.) O Kuntze. *The Journal of Horticultural Science and Biotechnology*, 81, 883-890.
- Song, K., Yim, W. C., & Lee, B. M. (2017). Expression of heat shock proteins by heat stress in soybean. *Plant Breeding and Biotechnology*, 5(4), 344-353.
- Tahir, S. M., Victor, K., & Abdulkadir, S. (2011). The effect of 2,4-Dichlorophenoxy acetic acid (2,4-D) concentration on callus induction in sugarcane (*Saccharum officinarum*). *Nigerian Journal of Basic & Applied Science*, 19(2), 213-217.
- Tonietto, A., Sato, J. H., Teixeira, J. B., de Souza, E. M., Pedrosa, F. O., Franco, O. L., & Mehta, A. (2012). Proteomic analysis of developing somatic embryos of coffee arabica. *Plant Molecular Biology Reporter*, 30(6), 1393–1399.
- Usman, M. G., Rafii, M. Y., Martini, M. Y., Yusuf, O. A., Ismail, M. R., & Miah, G. (2017). Molecular analysis of Hsp70 mechanisms in plants and their function in response to stress. *Biotechnology & Genetic Engineering Reviews*, 33(1), 26–39.
- Vitamvas, P., Urban, M. O., Skodacek, Z., Sosova, K., Pitelkova, I., Vitamvas, J., Renaut, J., & Prasil, I. T. (2015). Quantitative analysis of proteome extracted from barley crowns grown under different drought conditions. *Frontiers in Plant Science*, 6, 1-18.
- Wang, W. Q., Moller, I. M., & Song, S. Q. (2012). Proteomic analysis of embryonic axis of *Pisum sativum* seeds during germination and identification of proteins associated with loss of desiccation tolerance. *Journal of Proteomics*, 77, 68-86.
- Wang, M., Zou, Z., Li, Q., Sun, K., Chen, X., & Li, X. (2017). The CsHSP17.2 molecular chaperone is essential for thermo tolerance in *Camellia sinensis*. *Scientific Reports*, 7(1), 1237
- Xie, C., Zhang, R. X., Qu, Y. T., Miao, Z. Y., Zhang, Y. Q., & Shen, X. Y. (2012). Overexpression of *MtCAS31* enhances drought tolerance in transgenic *Arabidopsis* by reducing stomatal density. *New Phytologist*, 195(1), 124–135.
- Xu, Q., Ni, H., Chen, Q., Sun, F., Zhou, T., Lan, Y., & Zhou, Y. (2013). Comparative proteomic analysis reveals the cross-talk between the responses induced by H₂O₂ and by long-term rice black-streaked dwarf virus infection in rice. *Plos One*, 8(11), 1-14
- Zhao, P., Wang, D., Wang, R., Kong, N., Zhang, C., Yang, C., Wu, W., Ma, H., & Chen, Q. (2018). Genome-wide analysis of the potato Hsp20 gene family: identification, genomic organization and expression profiles in response to heat stress. *BMC Genomics*, 19, 61.
- Zhen, Y., Zhao, Z. Z., Zheng, R. H., & Shi, J. (2012). Proteomic analysis of early seed development in *Pinus massoniana* L. *Plant Physiology & Biochemistry*, 54, 97-104.
- Zhou, L., Xu, H., Mischke, S., Meinhard, L. W., Zhang, D., Zhu, X., Li, X., & Fang, W. (2014). Exogenous abscisic acid significantly affects proteome in tea plant (*Camellia sinensis*) exposed to drought stress. *Horticulturae Research*, 1, 14029-14037.
- Zhou, Q., Chen, Z., Lee, J., Li, X., & Sun, W. (2017). Proteomic analysis of tea plants (*Camellia sinensis*) with purple young shoots during leaf development. *Plos One*, 12(5), 1-18.