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Biochemical Changes in Embryogenic and Nonembryonic Callus of *Glycyrrhiza glabra* L. during Somatic Embryogenesis

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ABSTRACT

The continuous extraction of glycyrrhizin from *Glycyrrhiza glabra* L. has led to the critical endangerment of this therapeutic plant. Glycyrrhizin, a triterpenoid saponin, is a vital secondary metabolite renowned for its diverse pharmacological benefits, including anti-inflammatory, immunomodulatory, antiallergic, antiulcer properties, and even efficacy against HIV. However, the relentless harvesting of roots for high-value glycyrrhizin results in the obliteration of the entire plant, posing a severe threat to its existence and causing a detrimental impact on biodiversity. To counteract this challenge and conserve the species, sustainable approaches are imperative. One such approach involves the utilization of *in vitro* regeneration through somatic embryogenesis for clonal proliferation and genetic modification. This report presents findings on SE-associated proteins in *Glycyrrhiza glabra* L., accomplished through the implementation of the discontinuous gel electrophoresis technique. The study focused on a comparative isozyme analysis employing non-embryogenic and embryogenic calli of *G. glabra* L. Remarkably, these isozymes were exclusively identified and exhibited affiliations with a spectrum of cellular functions. A substantial portion of these proteins are engaged in a diverse range of metabolic and stress-related functions. This study utilized crude enzyme/total protein extracts as a basis to evaluate the genetic stability of *in vitro* cultivated plant tissue. Acid phosphatase, esterase, and peroxidase were selected as indicators for this assessment. Intriguingly, esterase and acid phosphatase exhibited significant polymorphism between the segregated embryogenic callus (EC) and non-embryogenic callus (NEC) derived from the same *G. glabra* L. genotype. Comparative analyses encompassing esterase, acid phosphatase, and peroxidase activities, alongside the expression profiles of these isozymes throughout somatic embryogenesis (SE), were conducted using polyacrylamide gel electrophoresis. Notably, the isoenzyme patterns of calluses were assessed. The activity of isozymes within an embryogenic line of callus surpassed that within a non-embryogenic counterpart. However, no conspicuous alterations in quality were observed between the non-embryogenic and embryogenic calluses.

Keywords *Glycyrrhiza glabra* L., Somatic embryogenesis, Embryogenic callus, Regenerating callus, Isoenzymes.

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INTRODUCTION

The medicinal plant *Glycyrrhiza glabra* L., known for its therapeutic properties, faces a critical risk of extinction due to ongoing glycyrrhizin extraction. *Glycyrrhiza glabra* Linn. (Family: Fabaceae) is a well-known medicinal plant used in traditional medicine across the globe because of its ethnopharmacological value for treating various ailments. The roots and rhizomes are the main medicinal parts of licorice. *G. glabra* Linn. is also called Liquorice, Mulaithi, or Yashtimadu. *Glycyrrhiza* derives from the ancient Greek term 'glykos', Sweet, and 'rhiza', meaning root (Thakur and Raj 2017). The primary active component, glycyrrhizin, serves as a commercial non-nutritional sweetener and flavor enhancer in a range of confectioneries and pharmaceuticals. This compound is predominantly found within the cylindrical-shaped roots and rhizomes, as documented by Badkhane *et al.* (2014). Another noteworthy secondary metabolite, the triterpenoid saponin glycyrrhizin, boasts a diverse array of pharmacological attributes, encompassing anti-inflammatory, immunomodulatory, antiallergic, and anti-ulcer properties. Moreover, it has demonstrated efficacy against HIV. The procurement of the invaluable glycyrrhizin entails uprooting the roots, culminating in the destruction of the entire plant. This practice engenders a formidable existential risk to the species and significantly impacts biodiversity.

The feasibility and potential for generating secondary metabolites through plant tissue culture have captured the attention of researchers worldwide. Cultures of plant cells, tissues, and organs provide a consistent and controlled avenue for the production of secondary metabolites, catering to market demand throughout the year. Somatic embryogenesis, commonly referred to as asexual embryogenesis, signifies the emergence of embryos from cells not stemming from gamete fusion. Consequently, the potential arises to generate numerous plantlets exhibiting complete similarity to the parent plant. This phenomenon facilitates the extensive proliferation of superior-quality clones, as evidenced by Campos *et al.* (2017). In commercial applications, Somatic Embryogenesis is being investigated as a strategy for vegetative propagation, as highlighted by Wójcikowska and Gaj (2015). Unraveling the intricacies underlying somatic embryogenesis (SE) holds substantial interest, as this phenomenon necessitates a redirection of cellular destiny, as posited by Elhiti (2013). Emerging evidence indicates that somatic embryogenesis involves an intricate network of signaling events and extensive transcriptional reprogramming, as indicated by recent research by Wang *et al.* (2020). Somatic embryogenesis constitutes a multifaceted phenomenon arising from a cascade of physiological, biochemical, and molecular transformations that unfold within plant cells. This intricate process hinges upon the attainment of embryogenic competence, encompassing dedifferentiation, chromatin reconfiguration, gene expression modulation, and aforementioned stress-induced events, as outlined by Krishnan and Siril (2017). *In vitro* treatment of cultured tissues with auxins induces a comprehensive transcriptomic reprogramming of somatic cells, orchestrating the modulation of numerous transcription factor genes associated with somatic embryogenesis. This fact is corroborated by the findings of Wójcik *et al.* (2020), Li *et al.* (2022), and Karami *et al.* (2023). Standard morphological indicators are insufficient for the identification and assessing the genetic uniformity of regenerated plants since they are influenced by environmental factors (Sugandh Suman *et al.* 2015). Isozyme electrophoretic pattern offers a reliable biochemical marker that is independent of environmental factors. Variation in the isoelectric points of the distinct monomers comprising an isozyme enables their separation through electrophoresis. Hence, delving into the foundational mechanisms governing differentiation across diverse developmental stages stands to benefit significantly from the scrutiny of isoenzymes. Furthermore, the evaluation of is oenzymes has been instrumental in probing the efficacy of techniques related to tissue culture and plant transformation. The enzymatic activities linked to the somatic embryogenesis process hold the potential to serve as viable biochemical markers. Notably, peroxidase activity and isozyme expression in non-embryogenic callus unveil an irregular oxidative stress landscape, a notion affirmed by the research of Rajput *et al.* (2023). The induction of embryo identity in somatic explants does not require endogenous auxin biosynthesis, whereas an increase in endogenous auxin levels is essential for maintaining embryo identity and in conjunction with auxin transport, promoting the development

of embryonic cells into histodifferentiated somatic embryos by Karami *et. al.* (2023).

The current biochemical analysis was conducted to detect potential variations or alterations in the quantitative and qualitative patterns of isoenzymes, specifically Acid phosphatase, Esterase, and Peroxidase, across various *Glycyrrhiza glabra* L. tissue types.

MATERIALS AND METHODS

Callus induction

From a leaf explant, Regenerating Calli, Embryogenic Calli, and non-Embryogenic Calli were initiated in *G.glabra* L. The leaf explant was excised and placed onto Murashige and Skoog (MS) medium (Murashige 1962) supplemented with 1.5 mgl^{-1} of 2,4-D. After a month in culture, the excised explant underwent development into a compact and light green callus. This tripartite callus was subsequently treated using Murashige and Skoog (MS) media, which were supplemented with a range of concentrations and combinations of auxin and cytokinin. These particular combinations have been previously established as effective for promoting organogenetic regeneration. After 4 weeks, the original calli were transferred to a 2,4-D-free medium, resulting in a transformation into spherical structures through subculture. Subsequently, these globular calli were subcultured in an agar-free medium devoid of 2,4-D, leading to their progression into somatic embryos. The third form of callus was maintained in an identical medium without additional attributes. The fact that auxin is widely used as an exogenous inducer and that endogenous auxin is necessary to facilitate efficient somatic embryo formation indicates the significance of auxin for *in vitro* somatic embryogenesis. These three types of calli were employed in the present investigation of isozyme profiles.

Isozyme profile in regenerating callus, embryogenic callus, and non-embryogenic callus of *Glycyrrhiza glabra* L.

Within the context of *Glycyrrhiza glabra* L., the focus of the examination encompassed regenerating callus, embryogenic callus, and non-embryogenic callus.

The process of differentiation arises from specific biochemical and physiological alterations triggered by plant growth regulators. Hence, significant variations in biochemical parameters were observed during somatic embryogenesis, which could be used as markers for monitoring the different events taking place during the process of somatic embryogenesis as underscored by Bilal *et al.* (2016). Isozyme electrophoresis was conducted within an electrophoresis unit, employing a discontinuous buffer system. The glass plates were separated, and the gel was subsequently immersed in a specific buffer. Distinct buffers tailored to each isozyme were utilized for immersing the gel, followed by the application of staining procedures. The composition of buffers and the staining protocol were individually customized for each enzyme. The staining protocols specific to each enzyme were executed. Upon distinct bands or zones becoming distinctly visible, the gels underwent fixation in a solution of methanol, water, and glacial acetic acid (in a ratio of 5:4:1) and were subsequently subjected to photographic documentation. Further analysis involved scanning the gels utilizing a Laser Densitometer (model LKB 2202 ultrascan) to determine the precise positioning of the isozyme bands or zones. The evaluation of relative mobilities (Rf) was also accomplished, involving the calculation of these values through the measurement of the distance migrated by a specific band with that of the bromophenol blue (tracking dye) front.

Acid phosphatase, esterase, peroxidase activity, and isoenzyme pattern

The visualization of acid phosphatase (EC 3.1.3.2; ACP) isoenzymes was achieved through gel electrophoresis, followed by staining of the gels using 1-naphthyl acid phosphate (sodium salt), Fast Garnet GBC, and a 0.15M acetate buffer (pH 5).

Esterase (EC 3.1.1.1; EST) isozymes were subjected to staining (Payne and Koszykowski 1978) solution consisting of 75 mg fast blue RR salt, 2.25 ml substrate solution (comprising 10 mg α-naphthyl acetate dissolved in 10 ml of 50% chilled acetone), within a 150 ml volume of 0.6 M phosphate buffer at a pH of 6.2.

The patterns of peroxidase (EC 1.11.1.7; PRX) isoenzymes were ascertained by employing polyacrylamide gel electrophoresis (PAGE) by the methodology outlined by Davis (1964). The visualization of these entities was achieved through the implementation of the Guaicol- H_2O_2 method, following the description outlined by Hislop and Stahmann (1971).

RESULTS AND DISCUSSION

Variation in protein content within *G. glabra* was observed through quantitative analysis using Lowry's method across diverse tissue samples. The specimens subjected to examination encompassed (a) Embryogenic Calli, (b) non-Embryogenic Calli and (c) Regenerating Calli. To assess the variability in directly regenerated shoots and to test the possibility for the isoenzymes to be used as markers in the assessment of the micropropagation effectiveness in the isoenzyme profiles of seven *R. aculeatus* by Ivanova *et al.* (2015).

In the current study, the isozyme profiles of Acid phosphatase exhibited distinct patterns, showcasing two discernible zones of activity designated as Zone A and Zone B. The electrophoresis lanes labeled 1, 2, and 3 contained buffer-soluble proteins derived from Regenerating Calli, Embryogenic Calli, and non-Embryogenic callus tissues, respectively. Notably, Zone A displayed a singular prominent band, identified as $AcpA_1$, with an Rf value of 0.208. This characteristic band was consistent across all three tissue types,

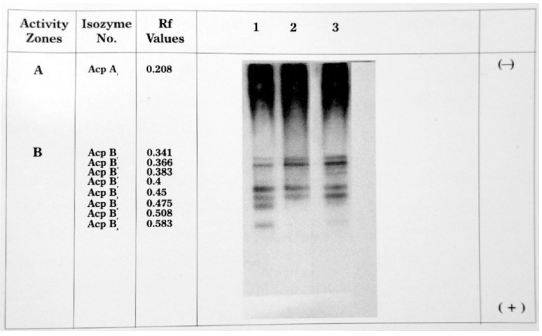

Fig. 1. The zymogram illustrates Acid phosphatase isoenzymes in different tissue samples of *Glycyrrhiza glabra*, obtained from regenerating callus (Lane-1), embryogenic callus (Lane-2), and non-embryogenic callus tissue (Lane-3). This visualization was achieved using PAGE (Polyacrylamide Gel Electrophoresis).

maintaining equal intensity levels. These observations were visually represented in (Fig. 1). Zone B exhibited a collection of eight distinct bands, each designated as follows: $AcpB_1$ (Rf 0.341), $AcpB_2$ (Rf 0.366), $AcpB_3$ (Rf 0.383), $AcpB_4$ (Rf 0.4), $AcpB_5$ (Rf 0.45), $AcpB_6$ (Rf 0.475), $AcpB_7$ (Rf 0.508), and $AcpB_8$ (Rf 0.583). Among these, $AcpB_3$ and $AcpB_4$ were solely present in Embryogenic Calli and non-Embryogenic Calli. Conversely, $AcpB_7$ and $AcpB_8$ exclusively manifested in organogenetic callus tissue. The respective intensities, Rf values, and activity zones were comprehensively tabulated for reference (Table 1). The results from this investigation revealed the presence of $AcpB_3$ and $AcpB_4$ (with Rf values of 0.383 and 0.4, respectively) in both embryogenic and non-embryogenic callus tissues. Although these tissue types exhibited similar zymogram patterns in terms of their quality, discernible quantitative differences were evident, highlighted by the greater intensity observed in non-embryogenic callus tissues (as illustrated in Fig. 1). Additionally, the regenerating callus tissues displayed the presence of $AcpB_7$ and $AcpB_8$ (with Rf values of 0.508 and 0.583, respectively, an observation depicted in (Fig. 1).

The enzyme patterns within various tissue types exhibited modifications throughout development and differentiation. These alterations were discerned through the appearance and subsequent disappearance of distinct bands. The selective activation of the genes responsible for synthesizing these isozymes during the developmental process is implied by these dynamic alterations in isozymes. Periodically shifting isozymes show that specific activation of certain isozyme-synthesizing genes occurs during development. The plants regenerated exhibit variation at the agromorphological, chemical, and molecular levels. Moreover, it should be possible to discern some of the somaclonal variations using agromorphological and genetically characterized RAPD markers. Thus, molecular changes can reflect stable changes in the genome that, introducing more variation in *C. winterianus* germplasm as indicated by Dey *et al*. (2015). The present investigation further highlights that regenerating callus tissues demonstrated an augmentation in Acid phosphatase activity, accompanied by the synthesis of multiple additional molecular forms of Acid phosphatase during the cytodifferentiation

Table 1. The zymogram pattern of Acid phosphatase observed across various tissues of *G. glabra.*

*Activity Zone	Isoenzyme no.	Rf values	RC	EC	NEC
A	Acp A_1	0.208	+++	+++	+++
B	Acp B_1	0.341	+	++	+++
	Acp B_2	0.366	++	+++	+++
	Acp B_3	0.383	–	+	++
	Acp B_4	0.4	–	+	++
	Acp B_5	0.45	++	++	++
	Acp B_6	0.475	+	+	++
	Acp B_7	0.508	++	–	–
	Acp B_8	0.583	++	–	–

The band's relative intensity is denoted by the (+) symbol, while the absence of the band is represented by the (-) symbol. *A= Cathodic Zone (-), B=Intermediate Zone.
Abbreviation:
RC- Regenerating Callus, Embryogenic Callus- EC, Non-embryogenic Callus-NEC.

Table 2. The zymogram pattern of Esterase observed across various tissues of *G. glabra.*

*Activity Zone	Isoenzyme no.	Rf value	EC	NEC	RC
A	Est A_1	0.16	++	+++	++
B	Est B_1	0.35	-	+	–
	Est B_2	0.45	-	++	++
	Est B_3	0.483	+++	–	–
	Est B_4	0.5	+++	++	++
	Est B_5	0.541	+++	–	+
	Est B_6	0.591	–	+	+

The band's relative intensity is denoted by the (+) symbol, while the absence of the band is represented by the (-) symbol. *A= Cathodic Zone (-), B=Intermediate Zone.
Abbreviation:
Embryogenic Callus- EC, Non-embryogenic Callus-NEC, RC-Regenerating Callus.

phase of the callus tissues.

Acid phosphatase is a common enzyme present in various cellular compartments, including the dictyosome, plastids, and interactions with the cell wall. From an agronomical point of view, as to how kinetic constants for the Acid phosphatase secreted "*in vivo*" by the roots of leguminous plants grown under P-deficiency or sufficiency, could be used as an early physiological indicator for P stress tolerance indicated by Jocelyne (2015).

Esterase isozymes

The isozyme profiles of Esterase exhibited two discernible zones of activity, as depicted in (Fig. 2), denoted as Zone A and Zone B. Within electrophoresis lanes 1, 2, and 3, buffer-soluble proteins were extracted from Embryogenic Calli, non-Embryogenic callus, and Regenerating Calli tissues, respectively. In Zone A, a singular prominent band named Est A_1, characterized by an Rf value of 0.16, was consistently observed across all three tissue types. However, notable variations in intensity were evident among the three tissues, as illustrated in (Fig. 2) and documented in (Table 2).

Zone B exhibited a collection of six discernible bands designated as Est B_1 (Rf 0.35), Est B_2 (Rf 0.45), Est B_3 (Rf 0.483), Est B_4 (Rf 0.5), Est B_5 (Rf 0.541), and Est B_6 (Rf 0.596), as displayed in (Fig. 2). A comprehensive presentation of the intensities, Rf values, and activity zones is compiled in tabular form within (Table 2). Notably, this study unveiled that Est B3 was exclusively present in the Esterase profile of embryogenic callus tissue, as visualized in (Fig. 2, Lane-1).

The investigation at hand has centered on the regeneration of complete plants from cultivated cells, constituting a focal area of study. Isozymes have emerged as a distinctive and finely tuned marker system, capable of shedding light on genetic and physiological transformations that precede cellular

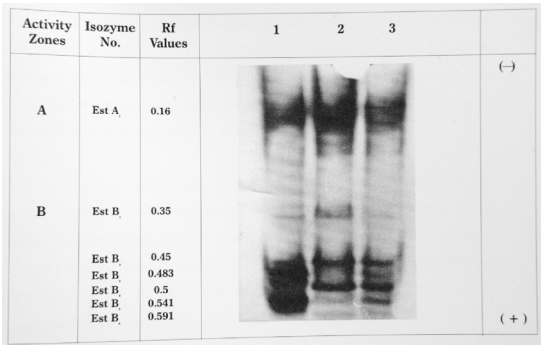

Fig. 2. The zymogram presents the Esterase isoenzymes observed in various tissue samples of *Glycyrrhiza glabra*. These samples were derived from embryogenic callus (Lane-1), non-embryogenic callus (Lane-2), and regenerating callus (Lane-3). The visualization of these isoenzymes was achieved through PAGE (Polyacrylamide Gel Electrophoresis).

differentiation within culture contexts. Notably, observations were made regarding the presence of specific Esterase enzymes within cultivated cells, with their activities showing an augmentation upon the depletion of auxins in cultures.

These findings exhibit a correlation with the cathodic isozymes highlighted in this study. Specifically, the embryogenic callus tissue (Lane 1, Fig. 2), grown in a growth regulator-free MS medium, displayed a broad and more intensely cathodic Esterase band. In contrast, non-embryogenic cells (Lane 2), which were cultivated in the presence of auxins, exhibited a comparatively less intense cathodic Esterase band. The molecular pathways that enable biological totipotency and coordinate somatic development. In the context of the current investigation, the cathodic Esterase $EstB_1$ (with an Rf value of 0.35) was exclusively detected within non-embryogenic callus tissue (Lane 2, Fig. 2). This finding strongly suggests the possibility that this cathodic Esterase, $EstB_1$, might be the unique Esterase. Therefore, it can serve effectively as a marker for identifying non-embryogenic callus, adding value to its potential application.

Peroxidase isozyme

The isozyme profiles of Peroxidase displayed dual activity zones (depicted in Fig. 3), categorized as Zone A and Zone B. Buffer-soluble protein extracted from non-embryogenic Calli, Embryogenic callus, and Regenerating Calli tissues were respectively loaded into Lanes 1, 2, and 3. Zone A featured five distinct bands with varying Rf values. Notably, $PrxA_1$ and $PrxA_2$ bands were consistently observed across all three tissue types, albeit with differing intensities. Within the intermediate zone, a solitary isozyme band with an Rf value of 0.645 was evident (as shown in Fig. 3). Comprehensive details of intensities, Rf values, and activity zones were systematically compiled within a tabular format (Table 3). Plant peroxidases have an important role in plant physiology including lignification and wound healing; these enzymes can also participate in the regulation of cell elongation. Peroxidases play a plant defense role against pathogens explained by Kaur *et al.* (2022).

In the context of the ongoing research, it was observed that embryogenic callus tissue (depicted in Fig. 3, Lane-2) exhibited notably elevated levels of Peroxidase activity in comparison to non-Embryogenic Calli. It has been posited that the activity of Peroxidase is under the influence of auxins. Previous research has put forth the notion of a regulatory function played by 2,4-D in governing Peroxidase activity. Additionally, their investigations led to the identification of a qualitative shift in the isozyme pattern after the removal of 2,4-D. Within the scope of this study, cathodic Peroxidase activity demonstrated an augmentation in intensity during the progression of embryo development within a 2,4-D-free medium. This observation implies a potential correlation between Peroxidase activity and the intricate processes of tissue development and differentiation. Moreover, Peroxidase enzymes have been proposed as prospective biochemical markers of somatic embryogenesis, encompassing both quantitative and qualitative dimensions. This assertion is supported by the research conducted by Oulbi *et al.* (2021), who investigated the expression of Peroxidase enzymes in two distinct types of explants sourced from two Moroccan olive cultivars.

Within higher plants, a range of Peroxidases exist, with their extraction patterns displaying specificity towards organs, developmental stages, and environmental conditions. In a proteomic analysis involving embryogenic calli (ECs) and non-embryogenic calli (NECs) within maize, Varhaníková *et al.* (2014) postulated an association between increased Peroxidase (POX) activity and the acquisition of regeneration capacity. However, contrasting instances exist where reduced POX activity is emblematic of ECs. A comparable decline in POX activity, particularly ascorbate peroxidase, was observed during the induction of regeneration in Avena nuda, as evidenced by Kohaich and Baaziz (2015). Aboulila (2016) documented the two different isozymes; peroxidase (PRX) and α naphthyl acetate esterase (EST) were used and analyzed to determine the genetic variability among the regenerated plants. The two analyzed isozymes successively showed polymorphic variations among the parent and 98 sweet potato plants regenerated from the three different callus induction media. Notably, the expression patterns of these isoforms exhibit diversity across distinct healthy plant tissues and are

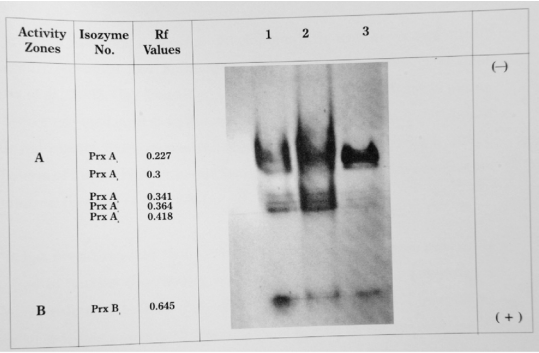

Fig. 3. The zymogram presents the Peroxidase isoenzymes observed in various tissue samples of *Glycyrrhiza glabra.* These samples were derived from non-embryogenic callus (Lane-1), embryogenic callus (Lane-2), and regenerating callus (Lane-3). The visualization of these isoenzymes was achieved through PAGE (Polyacrylamide Gel Electrophoresis).

Table 3. The zymogram pattern of Peroxidase observed across various tissues of *G. glabra.*

*Activity Zone	Isoenzyme no.	Rf value	NEC	EC	RC
A	Prx A_1	0.227	++	++	+++
	Prx A_2	0.3	-	-	+
	Prx A_3	0.341	++	++	–
	Prx A_4	0.364	+++	+++	-
	Prx A_5	0.418	+++	+++	-
B	Prx B_1	0.645	+	+	+

The band's relative intensity is denoted by the (+) symbol, while the absence of the band is represented by the (-) symbol. *A= Cathodic Zone (-), B=Intermediate Zone.
Abbreviation:
Nonembryogenic Callus- NEC, Embryogenic Callus-EC, RC-Regenerating Callus

modulated by developmental processes while also being susceptible to the influence of environmental factors. Moreover, the observation of varying Peroxidase isoenzyme bands, each exhibiting different intensities within the Calli of *Glycyrrhiza*, signifies the likelihood that these specific Peroxidase isoenzymes play targeted physiological roles within specific plant organs. The observed rise in activity within the embryogenic callus may be attributed to the pivotal role played by Peroxidase in the process of embryo cell wall formation. Intriguingly, a recent study focusing on embryogenic calli (EC) and non-embryogenic calli (NEC) underscores elevated peroxidase activity in the former compared to the latter, particularly during *in vitro* somatic embryogenesis in *Olea europaea.* This study advocates for the recognition of peroxidase as a potential marker for somatic embryogenesis, as proposed by Oulbi *et al.* (2021). The presence of Peroxidase within the soluble fraction, signifying oxidative status and reflecting a correlation with the process of differentiation, demonstrated heightened levels in calluses with bud-forming capabilities, cultivated across various auxin treatments. This elevation in peroxidase activity suggests that the biological system is undergoing conditions of tumultuous stress due to the generation of reactive oxygen species (ROS), as elucidated by Sachdev *et al.* (2021). This 'chaotic situation' could manifest as a transitory phase preceding the emergence or establishment of a new coherent biological system, such as somatic embryogenesis or organogenesis. Embryogenic Callus exhibited less peroxidase isozymes than Non-Embryogenic Callus. Somatic embryogenesis is an epigenetically regulated process that leads to the expression of enzymes involved in primary metabolism in Embryogenic Callus and secondary metabolism in Non-Embryogenic Callus. Peroxidase activity and isozyme expression in Non-Embryogenic Callus express a disorderly oxidative stress scenario as proposed by Rajput *et al.*(2023). This transitional state might account for the fluctuations observed in peroxidase activity or its isozymes across multiple research investigations.

In conclusion, the current trajectory of glycyrrhizin extraction has placed *Glycyrrhiza glabra* L. at a critical crossroads. Immediate action is required to shift towards sustainable practices that prioritize the preservation of this valuable plant. The integration of *in vitro* regeneration techniques offers a promising avenue to meet the demand for glycyrrhizin while safeguarding the plant's future and preserving biodiversity.

The assessment of whether tissue culture-derived plantlets possess identical genetic compositions to their parent plants was conducted through the examination of Isoenzymes, specifically Acid phosphatase, Esterase, and Peroxidase. The isolation of Embryogenic Calli (EC) and non-Embryogenic Calli (NEC) from *G.glabra* presents a valuable avenue to explore both the physiological and morphological mecha-

nisms, alongside biochemical aspects, of somatic embryogenesis (SE). This investigation holds promise in shedding light on the underlying biochemical transformations taking place during SE, along with identifying the contributing factors that influence the potential for successful SE. Esterase and Peroxidase have demonstrated a robust correlation with the progression of somatic embryogenesis (SE) and could potentially serve as valuable biochemical markers for this process. Conversely, the regenerating callus tissue exhibited an elevated Acid phosphatase activity, concomitant with the synthesis of an additional diverse molecular form of Acid phosphatase during the cytodifferentiation phase of callus tissues.

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REFERENCES

Aboulila AA (2016) Molecular genetic diversity and efficient plant regeneration system via somatic embryogenesis in Sweet potato (*Ipomoea batatas* (L.) Lam.). *Egypt J Genet Cytol* 45: 347-365.

Badkhane Y, Yadav AS, Bajaj A, Sharma AK, Raghuwanshi DK (2014) *Glycyrrhiza glabra* L. is a miracle medicinal herb. *Indo American J Pharmaceutical Res* 4: 5808-5816.

Bilal HA, Huma A, Buhara Y, Sabahat S, Khalid R, Mubarak AK (2016) Evaluation of biochemical markers during somatic embryogenesis in *Silybum marianum* L. *3 Biotech* 6: 71 https://doi 10.1007/s13205-016-0366-1

Campos NA, Panis B, Carpentier SC (2017) Somatic embryogenesis in coffee: The evolution of biotechnology and the integration of omics technologies offer great opportunities. Front *Pl Sci* 8:1460. https://doi.org/10.3389/fpls.2017.01460

Davis BJ, (1964) Disc electrophoresis- methods and application to human serum protein. *Ann NY Acad Sci* 121: 404-427.

Dey T, Saha S, Ghosh PD (2015) Somaclonal variation among somatic embryo derived plants —Evaluation of agronomically important somaclones and detection of genetic changes by RAPD in *Cymbopogon winterianus. South African J Botany* 96:112–121. http://dx.doi.org/10.1016/j.sajb.2014.10.010

Elhiti M (2013) Function of type-2 *Arabidopsis hemoglobin* in the auxin-mediated formation of embryogenic cells during morphogenesis. *Plant J* 74 (6): 946-958. https://doi.org/10.1111/tpj.12181.

Hislop EC, Stahmann MA (1971) Peroxidase and ethylene production by barley leaves infected with *Erysiphe graminis* f. sp. f. sp. hardei. *Physiol Plant Pathology* 1: 297-312. https://doi.org/10.1016/0048-4059(71)90050-6

Ivanova T, Angelov G, Bosseva Y, Gussev Ch, Dimitrova D, Stoeva T (2015) Isozyme markers for assessment of *in vitro* plants from *Ruscus aculeatus* L. *Trakia J Sci* 13 (2): 329-333. https://doi:10.15547/tjs.2015.s.02.070

Jocelyne A (2015) Acid Phosphatase Kinetics as a Physiological Tool for Assessing Crop Adaptability to Phosphorus Deficiency. Plants for the Future: Edited by Hany El-Shemy https://doi: 10.5772/60975

Karami O, Philipsen C, Rahimi A, Nurillah AR, Boutilier K, Offringa R (2023) *Endogenous auxin* maintains embryonic cell identity and promotes somatic embryo development in *Arabidopsis Plant J* 113 : 7–22. https://doi: 10.1111/tpj.16024

Kaur S, Samota MK, Choudhary M, Choudhary M, Pandey AK, Sharma A, Thakur J (2022) How do plants defend themselves against pathogens-biochemical mechanisms and genetic interventions. Physiol Mol Biol Plants 28 (2):485–504. https://doi.org/10.1007/s12298-022-01146-y

Kohaich K, Baaziz M (2015) New investigations on the guaiacol peroxidase of Opuntia *Ficus indica* L. and its modulation by ascorbic acid and copper. Towards an optimization of quantitative and qualitative tests. J Mol Catal B Enzyme119: 26–32 https://doi.org/10.1016/j.molcatb.2015.05.013

Krishnan SRS, Siril EA (2017) Auxin and nutritional stress coupled somatic embryogenesis in *Oldenlandia umbelata* L. *Physiol Mol Biol Pl* 23: 471–475. https://doi.org/10.1007%2Fs12298-017-0425-z

Li M, Wrobel-Marek J, Heidmann I, Horstman A, Chen B, Reis R, Angenent GC, Boutilier K (2022) Auxin biosynthesis maintains embryo identity and growth during BABY BOOM-induced somatic embryogenesis. Plant Physiol 188: 1095–1110. https://doi.org/10.1093/plphys/kiab558

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin Phenol reagent. *J Biol Chem*193(1): 265-275. https://doi.org/10.1016/S0021-9258(19)52451-6

Murashige T (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Oulbi S, Kohaich K, Baaziz M, Belkoura I, Loutfi K (2021) Peroxidase Enzyme Fractions as Markers of Somatic Embryogenesis Capacities in Olive (*Olea europaea* L.). Plants 10(5): 901. https://doi.org/10.3390/plants10050901

Payne RC, Koszykowski TJ (1978) Esterase isozyme differences in seed extracts among soybean cultivars. *Crop Sci* 18: 557–559. https://doi.org/10.2135/ops1978.0011183X001800040007x

Rajput DS, Rathore TS, Ansari SA, Faisal M, Alatar AA, Abdel-Salam EM, Shahzad A (2023) Biochemical changes in embryogenic and non-embryogenic callus of *Bambusa nutans* Wall. during somatic embryogenesis. Plant Cell, Tissue and Organ Culture (PCTOC) http://dx.doi.org/10.1007/s11240-023-02559-7

Sachdev S, Ansari SA, Ansari MI, Fujita M, Hasanuzzaman M (2021) Abiotic Stress and Reactive Oxygen Species: Generation, Signaling, and Defense Mechanisms. Antioxidants 10(2): 277. https://doi.org/10.3390/antiox10020277

Suman S, Kumari R, Sharma VK, Kumar H (2015) Isozyme analysis-based genetic fidelity assessment of micropropagated banana plants. Journal of Applied and Natural Science 7 (2):

579 –584. https://doi.org/10.31018/jans.v7i2.648

Thakur AK, Raj P (2017) Pharmacological perspective of Glycyr rhiza glabra linn.: a mini-review. *J Anal Pharm Res* 5(5): 14–12. https://DOI: 10.15406/japlr.2017.05.00156

Varhaníková M, Uvackova L, Skultety L, Pretova A, Obert B, Hajduch M (2014) Comparative quantitative proteomic analysis of embryogenic and non-embryogenic calli in maize suggests the role of oxylipins in plant totipotency. Journal of Proteomics 104:57-65. https://doi.org/10.1016/j.jprot.2014.02.003

Wang FX, Shang GD, Wu LY, Xu ZG, Zhao XY, Wang JW (2020) Chromatin accessibility dynamics and a hierarchical transcriptional regulatory network structure for plant somatic embryogenesis. Developmental Cell 54 : 742– 757. https://doi.org/10.1016/j.devcel.2020.07.003

Wójcik AM, Wójcikowska B, Gaj MD (2020) Current perspectives on the auxin-mediated genetic network that controls the induction of somatic embryogenesis in plants. *Int J Mol Sci* 21(4): 1333. https://doi.org/10.3390/ijms21041333

Wójcikowska B, Gaj MD (2015) Leafy cotyledone2 mediated control of the endogenous hormone content: Implications for the induction of somatic embryogenesis in Arabidopsis. Plant Cell Tiss Org 121:255–258. https://doi.org/10.1007/s11240-014-0689-8