Expression of Somatic Embryogenesis Receptor Kinase (*SERK*) gene and its regulation under the influence of exogenous additives during *in vitro* somantic embryo development in medicinal plants

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Abstract:

Somatic embryogenesis is a process whereby a single cell or a group of cells are induced to form totipotent embryogenic cells. Somatic embryogenesis always served as a model system for studying the molecular mechanisms underlying the embryogenic developmental process. There is an upsurge of interest in scientists to explore the molecular understanding of embryogenesis and the involvement of different genes and proteins during this developmental process. Studies have shown that somatic embryogenesis is under a stringent coordinated control of some regulatory genes among which somatic embryogenesis receptor kinase (*SERK*) gene has claimed an important role. In recent time expression of *SERK* gene was identified in embryogenic cultures of many higher plants indicating its positive role in embryogenic development. Studying the impact of *SERK* gene on somatic as well as zygotic embryogenesis shall improve the understanding of the molecular events leading to the formation of embryogenic cultures. The review highlights the correlation of *SERK* gene expression during somatic embryogenesis process and diverse functions of *SERK* gene during developmental changes in plants.

Keywords: Somatic embryogenesis, *SERK*, *In vitro* regeneration, Receptor like kinase, Plant developm

1. Introduction:

Plants have always been served as potential source of food, fuel, bioactive constituents, phytochemicals, drugs and many other medicinal, industrial and agricultural raw materials. Among them, medicinal plants deserve special mention. Medicinal plants contain inherent active ingredients having therapeutic and nutritional values; so, they have been and will continue to be important for mankind. Plant tissue culture is a technique in which cells and tissue are grown in aseptic condition in a culture medium under controlled conditions of light, humidity and temperature**.** Methods of plant cell, tissue, callus and organ culture for the controlled production of secondary metabolites was first initiated in large scales in the late 1950s. *In vitro* callus culture is a cost effective and attractive process. *In vitro* plant tissue culture has gained increasing attention over past few decades for the production of transgenic plants in order to isolate important natural constituents. Regeneration of whole plant from excised plant parts can be achieved by various means among which most important are organogenesis and somatic embryogenesis [1]. Both organogenesis and somatic embryogenesis can be accomplished either directly or indirectly through an intervening callus phase [2]. *In vitro* organogenesis and somatic embryogenesis is performed by the application of proper nutritional and hormonal supplements under aseptic conditions [3]. *In vitro* regeneration of plants can be achieved by means of two alternative mechanisms namely, organogenesis and somatic embryogenesis. Somatic embryogenesis is a process of initiation and development of embryos from competent somatic cells or nongametic cells, which in turn grows into a complete plant. Somatic embryogenesis forms the basis of cellular totipotency in higher plants which mimics some of the events of zygotic embryogenesis and has always been served as an efficient tool for studying the molecular events of embryogenesis [4]. Somatic embryogenesis provides a valuable mean of producing large number of transgenic plants. *In vitro* somatic embryogenesis has been attempted by many researchers for different practical applications. The most attractive application of this *in vitro* regeneration process is the mass propagation of plants [5]. Levels of exogenously applied plant growth regulators greatly influence the development of embryogenic cultures. *In vitro* somatic embryogenesis can be achieved either directly from explants without the production of callus or indirectly with the formation of an intervening callus from which the embryos develop [6]. Biochemical and morphological changes occur throughout the developmental stages of somatic embryogenesis and is strictly dependent on the differential expression of some genes. Among them *SERK* (somatic embryogenesis receptor kinase) gene was first identified as the marker of somatic embryogenesis [7]. The protein product of *SERK* gene is a type of receptor like kinase (RLK) containing five leucine rich repeats (LRR) in its extracelluar domain.

Association of *SERK* gene with somatic embryogenesis has become evident in recent years. Some plant species are recalcitrant that is difficult to be regenerated into whole plant via embryogenesis. So the search for a molecular marker of plant embryogenesis is of utmost importance in modern plant biotechnology experiments. *SERK* can be used as a marker for distinguishing between embryogenic and non embryogenic cells in plants. This review encompasses recent understanding of the role of *SERK* gene during embryogenic development in plants.

2. Relation between Somatic embryogenesis and *SERK* (somatic embryogenesis receptor kinase) :

Somatic embryogenesis is a process in which a single somatic cell or a group of cells are induced to form embryogenic cells leading to the formation of complete embryos analogous to zygotic embryos. Somatic embryogenesis can occur directly from explants without the formation of an intervening phase or indirectly with the formation of an intervening callus phase. Somatic embryogenesis constitutes a series of molecular events leading to the formation of competent somatic cells to induce totipotent embryogenic cells which can further be regenerated into a complete plant. Somatic embryogenesis can be induced *in vitro* by supplementing specific types of plant growth regulators in the culture medium. Somatic embryogenesis was first reported by Steward, Mapes and Mears (1958) [8] in suspension culture in carrot and by Reinart (1959) [9] in carrot callus grown on semisolid agar medium. Molecular analysis revealed the involvement of Somatic Embryogenesis Receptor Kinase (*SERK*) gene in the process of somatic embryogenesis. SERK belongs to the receptor like kinase (RLK) family of proteins which have five leucine rich repeats in their extracellular domain. The first identified plant *SERK* gene was *DcSERK* from carrot, whose expression was detected during somatic embryogenesis [7]. A number of *SERK* genes have been identified so far in different monocot and dicot plants including five Arabidopsis *SERKs* (*AtSERK1-5*). Expression analysis of SERK at mRNA and protein level is important to study the role of SERK during embryogenic development. SERK is known to be tightly regulated and hence the effect of external additives is of utmost importance. Since then a number of higher plants have been used to induce somatic embryogenesis. Das (Pal) and Sen Raychaudhuri, (2001) [10]; Begum *et al.,* (2008) [11]; and Paul *et al.,* (2009) [12] reported somatic embryogenesis in *Plantago ovata, Vigna radiata* and *Momordica charantia* respectively. Development of embryogenic cultures from somatic cells is achieved only when a single or a group of somatic cells acquire competence to be converted into embryogenic

cells which in turn differentiate to form somatic embryos. Different structural and physiological factors are responsible for directing nonembryogenic explant cells into embryogenic cells, one of which is exposure of the explant cells to specific combination and concentration of plant growth regulators. Auxins are known to be responsible for the induction of somatic embryogenesis. Among different auxins the most frequently applied is 2, 4-dichlorophenoxy acetic acid (2,4-D) followed by naphthalene acetic acid (NAA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), picloram and dicamba. In case of cytokinins, N^6-benzylaminopurine (BAP) is mostly used followed by kinetin, zeatin (Z) and thidiazuron (TDZ) (Jiménez, 2001). According to Jiménez, (2001) [3] auxin concentration should be reduced for the development of somatic embryos because it has been shown that further growth of the embryos is inhibited with continuous use of auxins. Other than plant growth regulators many other factors like physiological, biochemical etc can help in the acquisition of embryogenic competence. Most important of all these factors is the expression of specialized genes which induce the signal cascade of somatic embryogenesis.

SERK gene has been first identified in embryogenic culture of *Daucus carota* [7]. After that a large number of *SERK* gene homologs have been isolated and identified in different plant species. Besides playing important role in somatic embryogenesis *SERK* genes possess a diverse range of functions in plant growth and development. Among many different genes isolated from embryogenic suspension culture of *Daucus carota* at various phases of somatic embryogenesis most important is the Somatic Embryogenesis Receptor Kinase or *SERK* gene. Since it is expressed in somatic embryogenic culture, it came to be known as *SERK*. *SERK* encodes for a protein which belongs to the receptor-like protein kinase (RLK) family. RLKs are involved in different signal transduction pathways during plant development. SERK falls in the category of leucine-rich repeat RLKs (LRR-RLKs) which comprises the largest group of RLKs in the plant kingdom. The protein product of *SERK* gene contains an N-terminal signal peptide (SP), an extracellular leucine zipper (LZ), a single transmembrane (TM) domain, five leucine rich repeats (LRRs) in its extracellular domain, a distinct serine-proline-proline (SPP) domain, a serine/threonine kinase domain and the C-terminal region. The leucine rich domain acts as a protein binding region. Potential N-glycosylation sites are present in the intracellular and LRR motif of SERK protein. Existence of a proline-rich region in between the extracellular LRR domain and the membrane spanning domain strongly resembles the characteristic feature of extensions [7]. The intracellular domain contains eleven sub-domains which is a characteristic feature of protein kinases. The C-terminal motif

helps in protein-protein interaction which in turn transduce signal for phosphorylation cascade [7]. SERK is also known to have autophosphorylation activity. The domains VI and VII are the serine/threonine kinase [13]. The LRR sequence of SERK shows high homology with the *Arabidopsis RLK5* [14] and *Arabidopsis ERECTA* genes [15]. Somatic embryogenesis receptor kinase (*SERK*) gene is known to play an important role in triggering embryogenic competence in plant cells [7]. The protein encoded by the *SERK* gene is a receptor like kinase (RLK) consisting of an N-terminal domain with five leucine rich repeats [16]. Leucine rich repeat containing RLKs comprise the largest class of plant RLKs having tandem repeats of approximately twenty four amino acids with conserved leucines. Expression of *SERK* gene was first identified in embryogenic cells of carrot (*DcSERK*) suspension cultures [7] and later association of *SERK* with somatic embryogenesis was described in *Arabidopsis* [17]. Although a single *SERK* gene has been reported in carrot but Shah *et al.* (2001) [18] demonstrated the presence of five different *SERK* genes (*AtSERK1, AtSERK2, AtSERK3, AtSERK4*, and *AtSERK5*) in *Arabidopsis* indicating that in *Arabidopsis, SERK* genes exist as a gene family. Expression of *SERK* during somatic embryogenesis has recently been studied in a wide variety of plants including *Dactylis* [19], maize [20], *M. truncatula* [21], sunflower [22], *Citrus unshiu* [23], *Theobroma cacao* [24], *Octea Catharinensis* [25], cyclamen [26] and rice [27]. Both embryogenic and non-embryogenic cultures were studied for the detection of *SERK* expression in many plant species. Though expression of *SERK* has been strongly correlated with embryogenic competence, but now it is evident that *SERK* plays diverse roles during developmental program in higher plants. In sunflower, *SERK* is known to induce both somatic embryogenesis and shoot organogenesis [22]. Involvement of *SERK* in zygotic embryogenesis has also been demonstrated by Hecht *et al.*, (2001) [17] in *Arabidopsis*. Nolan *et al.*, (2003) [21] correlated the upregulation of *SERK* with high auxin concentration in *Medicago truncatula* during somatic embryogenesis. Similar observation has been documented by Roy *et al.*, (2008) [28]. Whole mount *in situ* hybridization revealed the differential expression of *SERK* gene in *Dactylis glomerata* [19]. *SERK* is known to act as a signal molecule in response to biotic and abiotic stresses [24]. Role of *SERK* during apomixis in *Poa pratensis* was also established [29]. Recently *SERK* gene (*ZmSERK*) have been isolated from *Zea mays* during embryogenesis [30]. According to Hu *et al.*, 2005 [27] *SERK1* gene triggers a positive signal during somatic embryogenesis and helps in host defence response. *SERK* was found to be expressed in somatic embryogenic cells of banana and has been shown to encode a protein containing 628 amino acids showing 82% homology with reported *SERKs* of carrot, *Arabidopsis*, *Medicago*, maize, rice and

coconut [31]. *SERK1* is also involved in the regulation of organ separation in *Arabidopsis* flower [32]. Four different *SERK* genes were identified in *Rosa hybrida* which share homology with other reported *SERK* sequences. Kedong *et al*. (2011) [33] showed the presence of *SERK1* gene in *Rosa canina* during initiation of protocorm-like bodies. Three different *SERK* genes were isolated from wheat by Singla *et al.* (2008) [34]. Ma *et al.* (2012) [36] recently isolated a *SERK* gene (*AcSERK1*) from pineapple (Ananas comosus) embryogenic culture and have shown that *AcSERK1* is highly expressed during acquisition of embryogenic competence and formation of globular shaped embryo. Cueva *et al.* demonstrated that *SERK* expression is high in embryogenic calluses of *Cyrtochilum loxense* but after the complete maturation of the embryos *ClSERK* expression is decreased.

3. Effect of Exogenous Additives on Somatic Embryogenesis and Expression of SERK Gene and SERK Protein

A number of investigations have been carried out to study the effect of external physical or chemical factors on somatic embryogenesis in order to get improved quality of plants. Among the various additives used in plant tissue culture, casein hydrolysate (CH) and coconut water (CW) are most important ones. CH provides a useful source of mixture of amino acids for plant tissue culture. It can be used for tissue culture experiments due to its cost effectiveness. CH is generally added to culture media at a concentration of 0.05-5%. CW is an undefined medium which is composed of a mixture of different amino acids, nitrogenous compounds, inorganic elements, organic acids, sugars and their alcohols, vitamins and many other components. In addition to this CW is a good source of auxins and cytokinins. Positive effect of CW in shoot proliferation has been reported in many plant species. CW is generally used in a range of 5-20%. CW is widely used in tissue culture experiments of several plants. Das (Pal) and Sen Raychaudhuri (2001) [10] have showed that CH and CW imparts a positive effect in enhancing somatic embryogenesis in *Plantago ovata*. Ronchi et al. [37] have reported that amino acids proline and serine stimulates the number of somatic embryo formation when added in a wide range of concentrations in medium supplemented with auxins. Glutamine has been proposed to be effective in enhancing embryogenesis in date palm. It has been observed that with increasing concentration of glutamine yield of embryos are increased [38].

4. Conclusion:

In vitro somatic embryogenesis provides a unique system to examine the molecular mechanisms that induce somatic cells to form competent embryogenic cells. Early researches remained restricted to the comparative studies of expression pattern of genes and proteins during somatic as well as zygotic embryogenesis. Over the past few years, researchers have been able to demonstrate experimentally the molecular aspects of embryogenic developmental process in a wide variety of plant systems. Studies on *SERK* gene would therefore advance an improved understanding of embryogenesis and its positive application in modern biotechnological approaches for crop improvement.

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