Exploring the involvement of the somatic embryogenesis receptor kinase (SERK) gene family in olive somatic embryogenesis efficiency

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Summary

The establishment of **somatic embryogenesis** (SE) protocols can work as an alternative for propagation of several portuguese cultivars characterized by their high-quality oils, but affected by serious agronomic problems. Nevertheless, the efficiency of SE strongly depends on the complex interaction between numerous factors. Plant regeneration through SE involves changes in gene expression, but little is known about the genes expressed during SE in olive. **Somatic embryogenesis receptor kinase** (SERK) belongs to a small family of receptor-like kinases involved in signal transduction in various plant species. In the present research, the involvement of SERK members in SE efficiency was evaluated by using two olive somatic embryogenic lines, obtained from zygotic embryos of cv. 'Galega vulgar', with different competence to differentiate somatic embryos.

Methodology

Somatic Embryogenesis

▪ Somatic embryogenic cell lines characterized by different competence to differentiate somatic embryos (high and low competent) were used to investigate the role of SERK genes. Cell lines, achieved in solid olive cyclic embryogenic (ECO) medium (Fig. 1 – A), were transferred to liquid ECO medium and maintained for several subcultures.

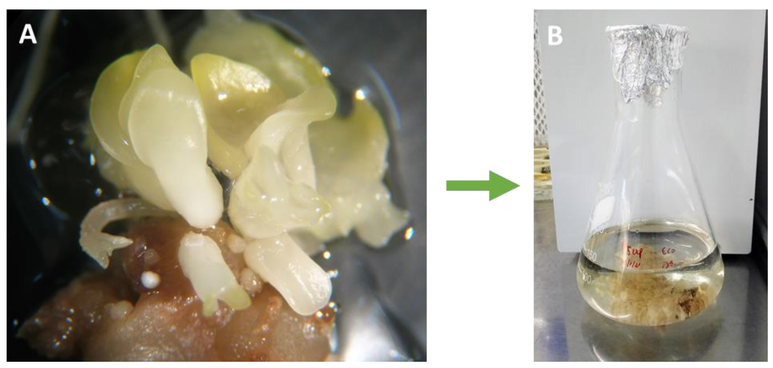
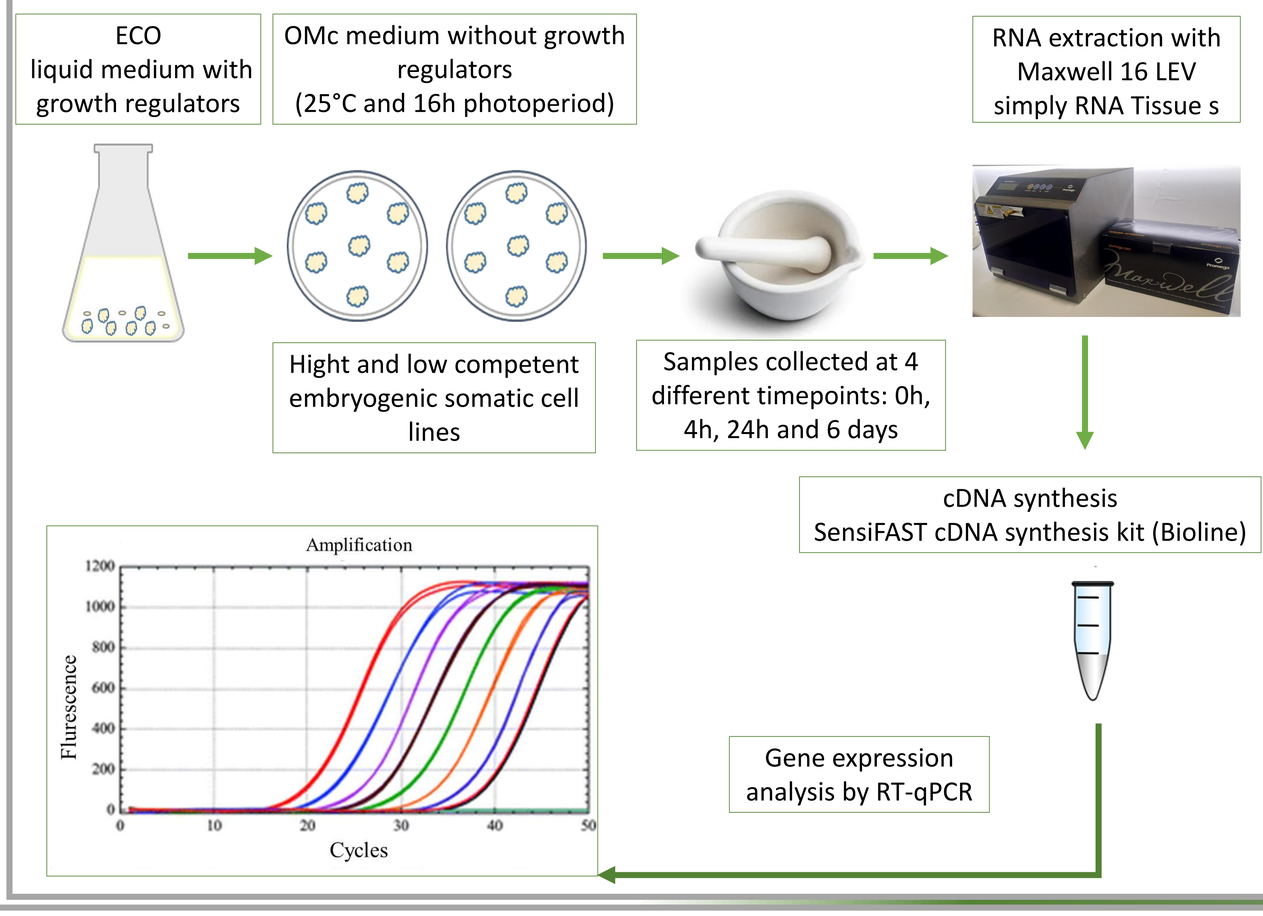


Figure 1. Somatic culture. (A) *Calli* with embryos in solid ECO medium and (B) somatic cell culture on liquid medium.

▪ After 3 weeks in liquid ECO medium (Fig. 1 –B), cell lines were transferred to OMc solid medium to promote differentiation of somatic embryos.

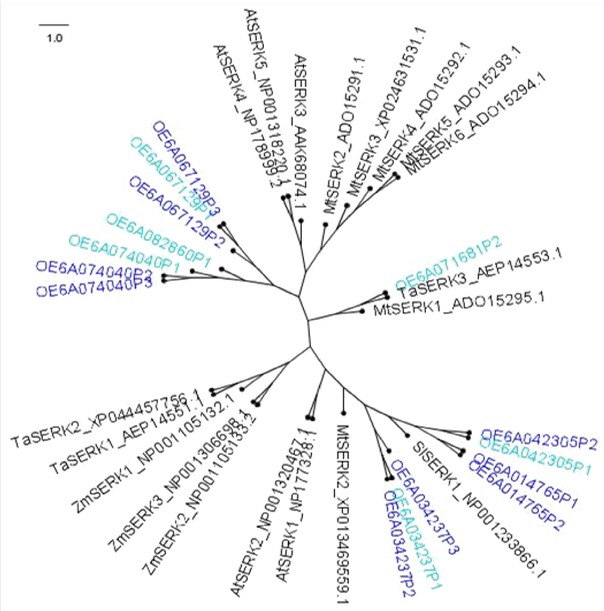
▪ Samples from both cell lines were collected at 0h, 4h, 24h, and 6 days and used for total RNA extraction and cDNA synthesis. The involvement of SERK gene family members was evaluated by reverse transcription quantitative real-time PCR (RT-qPCR).

Methodology Workflow



Results

1. Identification of *OeSERK* genes



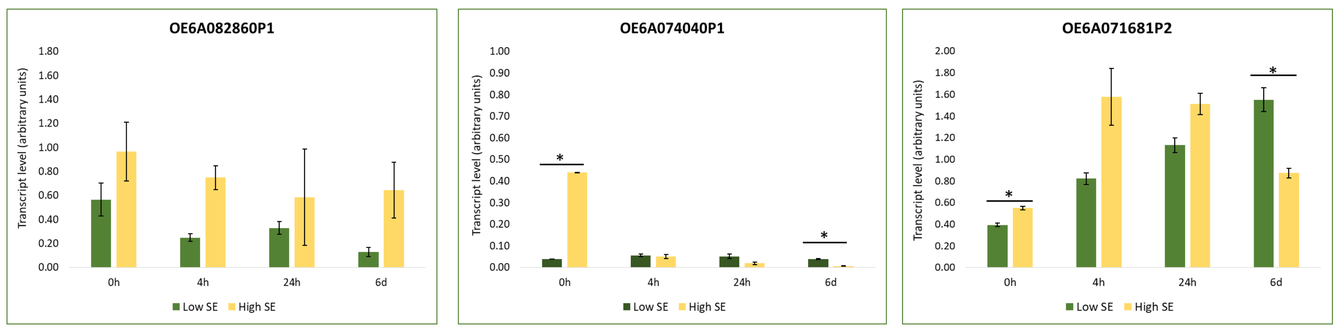
▪ Fifteen *OeSERK* sequences were identified at the olive genome databases.

▪ Phylogenetic relationships among deduced olive SERK proteins can be divided into 4 main branches (blue colored).

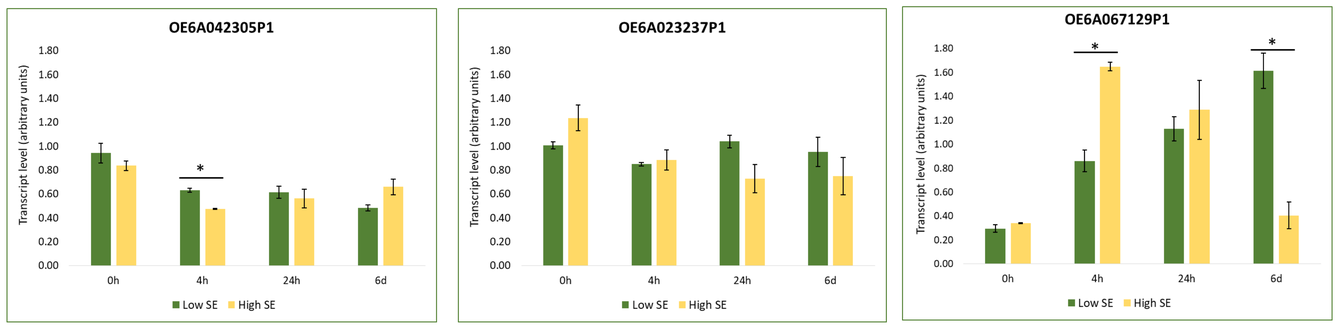
▪ For gene expression analysis it were considered six genes (principal transcript was considered for primers design).

Figure 2. Neighbor-Joining (NJ) tree showing the phylogenetic relationships among deduced olive SERK sequences and sequences from 5 plant species.

2. *OeSERK* expression analysis



- OE6A08860P1 presents a high transcript level in the high competent cell line during all time course.
- OE6A074040P1 and OE6A071681P2 present a significant high transcript accumulation in high competent cell lines at 0h, previously to inoculation of the expression medium (devoid of growth regulators).



- OE6A042305P1 exhibit a decrease at 4h in both cell lines with significant differences only here visible.
- OE6A023237P1 appears with a similar pattern of OE6A042305P1.
- The OE6A067129P1 presents a similar pattern of OE6A071681P2 showing the peak of expression at the early timepoint in the high competent cell line (4h).
- A negative regulation seems to exist between those both genes and the OE6A074040P1.

Final Considerations

- This work allowed to show the involvement of SERK genes in SE in olive;
- Significant differences were achieved for different genes when compared high and low competent cell lines;
- Further studies will be carried out to validate the data achieved at transcript level.

Acknowledgments

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