A) Proposed project, including introduction, materials and methods, tasks, deliverables, duration, pointing out the link/benefit to COST, mentioning if a joint application will result from it. Brief project description (max 1 page). Please indicate what impact this STSM will have for your project. How does the topic fit into the goals of the COST Action?

ABA and CK signaling in root-to-shoot communication to improve salt-tolerance in tomato

Work Plan Summary

This study will determine the involvement of ABA and CKs and their interaction in the physiological and agronomical responses of tomato to salt stress. We will focus on the importance of the root system in long distance versus local signaling via hormone flow-modeling using a grafting approach. A functional experimental approach will modify both cytokinin and ABA concentrations in the domestic tomato. Tomato plants (*Solanum lycopersicum* L.) either overexpressing genes encoding enzymes involved in ABA (*NCED*) or cytokinin (*IPT*) synthesis will be used. Short-term (2 months, June-July) experiments through reciprocal grafting approaches examining vegetative growth will be conducted in hydroponics under fully-controlled environmental conditions (phytotron) and will focus on the impact of transgene expression on plant growth, biomass allocation and leaf senescence, in relation to the alteration of endogenous hormone levels in the leaves, xylem sap and roots. In parallel, long-term experiments will study the effect of different rootstock on hormone and ionomic fluxes in the plants and yield-related parameters (reproductive stage between May and July of ongoing experiments). Ionome status and xylem hormone concentrations (ABA, CKs, ACC, IAA and JA) will be determined and whole data collection will be analyzed through the stage.

Benefits to COST FA1204 and impact of the STSM on the projects

The position will be integrated in the Spanish national project '**Cytokinin interactions with ABA and K^+: A root-integrative approach for improving tomato (*Solanum lycopersicum* L.) salinity tolerance' (CICYT-FEDER AGL2011-27996, 2011-2014).** This project aims to understand the hormonal interactions between rootstocks and scions in order to improve salt tolerance in tomato through the exploitation of root-to-shoot signaling. These objectives are central to the WG2 (Rootstock-scion interactions and graft compatibility) and WG3 (Rootstock-mediated resistance to biotic and abiotic stresses) of the COST Action FA1204. This STSM will provide mutual benefits for the ongoing projects in both groups sharing the same objective of improving salt tolerance in tomato crop through grafting. Hence, it is likely that the complementary activities to approach a common objective will result in future joint applications, and certainly will help to achieve the objectives of the Action.

Introduction

Salinity limits plant productivity by modifying plant hormonal and nutritional balances. Abscisic acid (ABA) and cytokinins (CKs) are phytohormones that mediate plant response to abiotic stress and are mainly considered to be produced in the roots. Most studies devoted to the role of phytohormones in stress physiology have focused on the stress hormones such as ABA and ethylene and ignored other possible interactions. ABA and CKs have long been considered antagonists but the role of this antagonism in mediating

abiotic stress resistance has received scant attention. Despite much research on root-to-shoot signalling, it is uncertain whether shoot hormonal changes following salt stress are due to changes in root hormone export, or local metabolic changes in the shoot. The conflicting data, previously reported, about the role of root hormone supply and its physiological impact on the shoot suggests the role of root-synthesised CKs and ABA in regulating shoot responses requires re-evaluation. Our previous results revealed that selective *IPT* (an enzyme that catalyses the rate limiting step in CKs synthesis) overexpression only in the roots increased root and shoot bioactive CKs concentrations but also improved shoot growth of salinised tomatoes. This increased leaf CK concentration coincided with decreased leaf ABA concentrations. It is still unknown if the decreased ABA concentration of salinised *IPT* plants is due to a direct CK impact on ABA synthesis or catabolism. The availability of transgenics with a graded series of increased ABA (plants overexpressing *NCED* (cis-epoxycarotenoid dioxygenase) or CK concentrations, new high throughput techniques for hormonal and ionomic analysis, optimised assays for enzyme activities and ease of grafting in tomato now allows a multi-level (molecular / biochemical / physiological / agronomic) assessment of the significance of ABA/CK interactions in regulating root and shoot growth under salinity in order to improve salinity tolerance.

This project will determine the involvement and functions of the phytohormones abscisic acid (ABA) and cytokinins (CKs), and especially their interactions, in the physiological and agronomic responses of tomato to salt stress, by addressing the following questions:

1. Does rootstock overproduction of ABA or CKs improve plant growth and fruit yield under salinity?
2. Does rootstock overproduction of ABA or CKs modify systemic ABA / CK signalling under salt stress?

Plant Material and Methods

A functional experimental approach will modify both cytokinin and ABA concentrations in the domestic tomato. Three independent sets of tomato plants (*Solanum lycopersicum* L.) either overexpressing genes encoding enzymes involved in ABA (*NCED*) or cytokinin (*IPT*) synthesis will be used. *NCED* and *IPT* genes are expressed constitutively either under the control of the CaMV 35S promoter (*35S::IPT)* or the Gelvin superpromoter for (*NCED)* (2 lines: sp12 and sp5 which show leaf ABA concentrations 2.7 and 4.3 fold of WT) (Thompson et al., 2007). Additionally, a particular constitutive *35S::IPT* transformant will be used with a moderately (30%) higher leaf *trans*-zeatin concentration (compared to azygous plants) without apparent phenotypic alterations under control conditions (Ghanem et al., 2011).

Shoot and root growth, leaf senescence and fruit yield of the graft combinations will be measured at several salt concentrations in different sets of experiments (short-term and long-term). Short-term experiments through reciprocal grafting approaches examining vegetative growth (2 months, June-July) will be conducted in hydroponics under fully-controlled environmental conditions (phytotron) and will focus on the impact of transgene expression on plant growth, biomass allocation and leaf senescence, in relation to the alteration of endogenous hormone levels in the leaves, xylem sap and roots. In parallel, long-term experiments will study the effect of different rootstock on hormone and ionomic fluxes in the plants and yield-related parameters (ongoing experiments, the candidate will intermittently contribute to analyse the plants at reproductive stage between May and July).

Task 1: Assessing the importance of the root system in long-distance versus local signalling via hormone flow-modelling

Grafting allows the role of long-distance CK and ABA signalling in mediating shoot physiological responses and hormonal concentrations to be assessed. Wild-type tomato seedlings will be either self-grafted or grafted on selected transgenic lines (overexpressing the *IPT and NCED* genes) as rootstocks. Reciprocal grafting between different transformants will result in 9 rootstock-scion combinations (WT/WT - both within and between the different genetic backgrounds that the ipt/ NCED lines are available in, ipt/WT, WT/ipt, ipt/ipt, NCED/NCED, NCED/WT, WT/NCED, NCED/ipt, ipt/NCED). Selected physiological variables (growth, leaf senescence and water and nutritional status) will be analyzed during plant development in relation to the contributions of root-exported hormones and shoot hormonal synthesis to the changes in shoot ABA/CK concentration through detailed hormonal flow-modeling studies (as developed by Jiang and Hartung, 2008).

Deliverable: New information will be obtained about the root and shoot sourced hormones on the whole plant response to salinity and on ABA/CK interactions through reciprocal grafting.

Task 2: Assessing the long-term impact of ABA and CK changes on yield-related parameters under salt stress

To determine if altered CK and ABA accumulation could improve fruit yield of salinised plants, seedlings of the different transgenic lines will be used as rootstocks of a commercial tomato variety and grown under commercial greenhouse conditions. A standard fertilization mixture for tomato will be applied by a drip irrigation system. A field-representative moderate salt treatment (75 mM NaCl) will last for 6 months. Fruit yield, number and weight will be determined and actively growing (20 and 35 days after anthesis) and leaf xylem sap will be collected for hormonal concentrations analysis and sink strength/sink activity determination, according to Balibrea et al., (2003) and Albacete (2009). Physiological variables will be analyzed during crop development at vegetative and fruiting stages in order to explain the agronomical salt tolerance.

Deliverable: Yield experiments will represent the 'proof of concept' of the CK-ABA root-integrative approach on improving tomato salt tolerance.

References

- Albacete et al. 2009. Plant, Cell & Environment, 32: 928-938.
- Balibrea et al. 2003. *Physiologia Plantarum,* 118: 38-46.
- Ghanem, Albacete *et al.* 2011, *Journal of Experimental Botany* 62: 125-140
- Thompson, Andrews, *et al.* 2007, *Plant Physiology* 143:1905-1917.
- Jiang & Hartung, 2008. *Journal of Experimental Botany* 59:37–43.