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Specific nutrient absorption rates of transplanted cucumber seedlings are highly related to RGR and influenced by grafting method, AMF inoculation and salinity

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ABSTRACT

The objective of this study was to assess the influence of different grafting methods and mycorrhizal inoculation of (salt-stressed) cucumber seedlings regarding plant establishment and nutrient absorption rates. Young cucumber (*Cucumis sativus* L) plants were grafted onto AMF-free, or AMF inoculated *Cucurbita maxima* × *C. moschata* transplants, by two grafting methods; common root intact, splice grafting (SG) and root-pruned splice grafting (RPSG). A similar number of self-rooted cucumber plants were kept as control. The results of this study indicate that the RPSG method offers advantages over the SG method, especially under saline conditions by improving the specific absorption rates (SAR). Improved growth rates of RPSG plants could be related to significantly higher SAR of certain elements such as N, P (under non-saline conditions) and N, Ca, K, Mg and Fe (under salt stress). High SAR of RPSG seedlings are suggested to be largely based on the young adventitious roots established after pruning. The inoculation with AMF enhanced the nutrient uptake and stand establishment rate of cucumber seedlings, especially in salt stressed grafted plants, through extending their root system and enhancing the photosynthetic rate. The study indicates how stand establishment rate and plant nutrition can be enhanced in commercial cucumber production by using cutting grafting (RPSG) and pre transplanting AMF inoculation on favourable grafting combinations.

1. Introduction

Due to limited availability of arable land and the high market demand for vegetables worldwide, crops such as Cucurbits (cucumber, melon, and watermelon) are frequently cultivated under unfavourable conditions. Excess soil salinity is an increasing constrain for crop production, especially in irrigated and protected cultivation systems and in (semi-) arid areas. In the Mediterranean regions, 25% of irrigated agricultural land is significantly affecting by salinity (Daliakopoulos et al., 2016). The effects of excess soil salinity on plants involve (i) a reduced water availability, (ii) toxicity of excessive Na and Cl ion concentrations, and (iii) nutrient imbalance caused by a salt effect on nutrient uptake and/or transport (Grattan and Grieve, 1998; Evelin et al., 2009).

Finding measures to counteract the deleterious effects of salinity on plant is of utmost importance in commercial plant production. Grafting often improves the abiotic stress tolerance of fruit vegetables (Rivero et al., 2003; Edelstein et al., 2012; Rouphael et al., 2017) - beside the traditional use for increasing the disease resistance (Lee, 2007). Accordingly, an instant technique for avoiding or reducing yield losses in Cucurbitaceae caused by excess soil salinity is to graft high-yielding genotypes onto rootstocks capable of ameliorating salt-induced damage to the shoot (Rouphael et al., 2017). Indeed, grafted plants under saline conditions sustain a higher photosynthesis and leaf water content, a more balanced root-to-shoot ratio, and lower accumulation of Na^+ and/or Cl^- in shoots than non-grafted (NG) or self-grafted plants (Estañ et al., 2005; Huang et al., 2009; Colla et al., 2010). Nutrient uptake and translocation to the shoot are often improved in favourable grafting combinations under both saline and non-saline conditions (Rouphael et al., 2017; Savvas et al., 2017). However, a number of grafting methods is employed (Lee, 2007; Yassin and Hussen, 2015) and it is largely unknown if or how this is affecting salt tolerance of the grafting combinations in general and nutrient absorbance rates in specific. The root-pruned splice grafting method (RPSG; also known as cutting graft

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method) is increasingly used in the production of fruit vegetables, particularly among Cucurbitaceae. In this technique, scions are grafted onto stem cuttings received from the rootstock, which are subsequently transplanted into a growing medium for rooting (Shibuya et al., 2007). Therefore, different from common splice grafting (SG) methods where the root system remains intact, in RPSG new adventitious (shoot-born) roots develop; a detailed description can be found in Balliu and Sallaku (2017). Although, since a while, SG and RPSG methods are simultaneously used in commercial nurseries, the scientific evidences of differences between them in regard to stand establishment rate and nutrient absorption capacity under excess soil salinity are limited. Earlier reports on cucumber, watermelon and other crops showed that the new roots grew very fast, and at time of transplanting the dry matter of the root system in RPSG seedlings often matched that of intact roots of SG seedlings (Lee et al., 1998; Babaj et al., 2014). For watermelon there is first evidence that RPSG plants continue to grow faster than SG plants after transplanting and can better resist the negative effects of salinity (Balliu et al., 2014) - however, the underlying mechanisms remain largely unknown.

Besides grafting, the facilitation of mycorrhizal symbiosis, adding arbuscular mycorrhizal fungi (AMF) inocula either to seedlings' growing medium or into the planting hole at time of transplanting, is receiving increasing attention. AMF have been frequently reported to improve crop tolerance to abiotic stress including salinity (Kumar et al., 2014). The symbiosis of plants with AMF often results in increased nutrient uptake, accumulation of osmoregulatory compounds, an increase in photosynthetic rates, and a decrease in root respiration and water use (Al-Karaki, 2000; Porcel et al., 2012; Rewald et al., 2015). While AMF are among the most abundant organisms on earth, reports on reduced spore production and root colonization rates in saline soils (Kumar et al., 2014) suggest that crops can benefit from additional inoculation with AMF spores.

Uptake efficiency and transport depend on the morphology and physiology of the plants, especially the roots, and their mycorrhizal symbionts - which can both be altered by the use of different rootstocks and grafting methods (Porcel et al., 2012; Nawaz et al., 2016). High uptake efficiency is especially decisive under excess soil salinity where both Na and Cl ions hamper nutrient uptake while the overall C budget of plants is constricted by lower photosynthetic rates. For example: high Na^+ concentrations in the soil solution decrease the uptake efficiency of K^+ and Ca^{2+} and high Cl^- competes with the uptake of NO_3; the P nutrition of the plant can be affected even though there is no direct competition during uptake (Grattan and Grieve, 1998). Especially enhanced K^+ and decreased Na^+ accumulation by mycorrhizal symbionts and selected rootstocks, respectively, in saline soils is believed to help maintaining a high K/Na ratio - preventing e.g. the inhibition of proteins. However, although there are numerous studies regarding the advantages of grafted cucumber plants over non-grafted seedlings regarding growth and nutrient contents (Savvas et al., 2010; Colla et al., 2012; Huang et al., 2013a) information about differences induced by grafting methods are scarce (Babaj et al., 2014). In addition, even fewer studies (e.g. for tomato; Tüzel et al., 2012) addressed the combined effects of grafting and AMF inoculation on growth and yield of salt-stressed plants, yet and information about differences between grafting methods regarding plant-AMF interactions and nutrients uptake rates (under salinity) is virtually absent.

The objective of our three factorial approach was to assess the combined influence of grafting method (NG, RPSG, and SG) and AMF inoculation on (salt-stressed) cucumber seedlings regarding 1) growth and stand establishment rate and 2) plant nutrient status and specific nutrient absorption rates. Plant performance and nutrient uptake of grafted plants are compared to non-grafted seedlings. It is hypothesized that management methods at the nursery stage, which increase the nutrient uptake efficiency (i.e. a younger, more active root system and increased AMF symbionts), are especially beneficial to increase the performance of transplanted cucumber seedlings in saline soils. Considering the high significance of a fast and successful establishment of transplanted seedlings on further plant performance, the period of study was on purpose limited to only the immediate post transplanting period.

2. Materials and methods

2.1. Plant material and experimental set-up

The experiment was conducted during the spring of 2013 in a plastic film greenhouse located in Tirana, Albania (41°23′27″N, 19°39′18″E). Graded seeds of a commercial cucumber variety (*Cucumis sativus* L. cv. Ekron F_1) and of a commercial rootstock (*Cucurbita maxima* DUCHESNE EX POIR. × *C. moschata* (DUCHESNE EX LAM.), cv. Nimbus F_1) were sown in Styrofoam transplant trays (30 cm^3 volume of plugs). Trays were filled either with i) vermiculite (Agra-Vermiculite, Pull Rhenen B.V., The Netherlands) and 10% (vol/vol) crushed, expanded clay particles coating AM-fungal spores (~200 spores g^{-1}; mixture of *Claroideoglomus etunicatum, Funneliformis mosseae, F.geosporum, Rhizophagus clarum, R. irregularis*, names according to Schüßler and Walker (2010); AMF+), or ii) vermiculite with crushed, spore-free expanded clay particles (10% vol/vol; AMF). The clay particles with / without AMF spores were supplied by BioSym B.V. (Hengelo, Netherlands) and homogenously mixed with the vermiculite before sowing; both substrate types were saturated with a nutrient solution containing 1 g L^{-1}Terraflex T fertilizer (N,P_2O_5, K_2O of 18%,7%,25% + TE; ICL Fertilizers, Belgium). Plants were irrigated with tap water as required; leaching was minimized.

Fourteen days after sowing, 160 cucumber (*C. sativus* cv. Ekron) scions were grafted onto *C.maxima* × *C. moschata* cv. Nimbus rootstocks by two different grafting methods (80 plants each): the common splice-grafting (SG) and root pruning splice-grafting (RPSG). About 80 non-grafted (NG) cucumber plants were kept as control. Different from the root intact method (SG), RPSG-grafted cuttings were obtained by grafting scions on stem cuttings of the rootstock, which were subsequently transplanted into growing medium for rooting (Shibuya et al., 2007). Immediately after grafting, all plants were placed in a growth chamber (KBW 400, Keison Products, Essex, England). An air temperature of 26 °C and a RH of 100% was maintained for three days and then gradually decreased to 90%; PPFD was 100 µmol $m^{-2}s^{-1}$(white fluorescent lamps) with a photoperiod of 12 h. At the fourth day after grafting, plants were moved to a plastic film greenhouse until the end of experiment.

At DAG (day after grafting) 14, all plants were transplanted into 200 cm^3 plastic pots (7 cm high, 5.5 cm wide) filled with nutrient-saturated (1 g L^{-1},Terraflex T) vermiculite. Two different levels of salt-stress (0 and 80 mM NaCl) were imposed by the addition of sodium chloride (NaCl) to the nutrient solution. Non-inoculated (AMF) and inoculated (AMF+) seedlings were equally distributed to both salinity treatments. Thus, a full factorial design with the following factors; grafting method (NG, RPSG, SG), arbuscular mycorrhizal fungi (AMF-, AMF+) and salinity (0, 80 mM NaCl; after DAG 14) was established (n = 8). Plants were irrigated during DAG 14–24 with tap water as required; leaching was avoided.

2.2. Plant sampling for biomass and growth rate determination

Plant samples for biomass and growth rate determination were received in three successive intervals (respectively 7th, 14th, and 24th day) from the day of grafting, hereafter named as day after grafting (DAG). Each time, i.e., DAG 7 (data not shown), DAG 14, and DAG 24, eight plants of each treatment were selected randomly for harvest. Roots were gently rinsed, and plants were dissected and separated into roots, stems and leaves. The organs were subsequently dried (65 °C, 48 h) and the dry matter (DM_{Root}, DM_{Stem}, DM_{Leaves}) was determined separately to an accuracy of ± 1 mg (TP 303; Denver Instruments