

## Breeding for improved responsiveness to arbuscular mycorrhizal fungi in onion

G.A. Galvan<sup>1,2</sup>, K. Burger-Meijer<sup>1</sup>, Th.W. Kuiper<sup>3</sup>, C. Kik<sup>4</sup> and O.E. Scholten<sup>1</sup>

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### Abstract

Arbuscular mycorrhizal fungi (AMF) play an important role in the uptake of nutrients and water from soil. Onions, *Allium cepa* L., are plants with a shallow root system. As a result, onion plants need a lot of fertilizer for their growth. Furthermore, onion plants are sensitive to drought. The aim of the current research project is to study the beneficial effect of mycorrhizal fungi on the growth and development of *Allium* species and to determine whether it is possible to improve onions for mycorrhizal responsiveness by means of breeding. Variation among *Allium* species and segregation observed in a interspecific tri-hybrid population indicate that selection and thus breeding for high responsiveness to AMF is possible.

### Introduction

Arbuscular mycorrhizal fungi (AMF) are fungi that occur naturally in soil. These fungi play an important role in plant growth since they contribute to the uptake of nutrients and water from soils (Ryan and Graham, 2002). Onion (*Allium cepa* L.) is one of the leading vegetable crops worldwide. Also in Europe, the crop is of considerable economic importance. The global distribution of onions is due to the universal acceptance for food and condiment, but certainly not due to its simplicity of growing. In fact, onion is a crop that is difficult to cultivate and one of the major challenges is to provide onion plants with sufficient nutrients (Brewster, 1994). For onions large amounts of fertilizer are needed. The sustainability of large fertilizer input is highly questioned and growing onions in low-input systems with reduced fertilizer inputs are gaining ground. For low-input systems, plants have to be good nutrient scavengers. Therefore, productivity and stability of onion production in such systems can be problematic (Greenwood et al, 1982). Onion root systems consist of superficial roots that are rarely branched and lack root hairs (Portas, 1973), making them inefficient in uptake capacity of water and nutrients such as phosphate (Wininger et al, 2003). A possible route to improve the uptake of water and nutrients in onions is to improve its root system. A wild relative of onion, *Allium fistulosum* L., is known for its extensive root system. Via a bridge cross with *Allium roylei* genes from *A. fistulosum* can be introgressed into onion germplasm as was shown by Khrustaleva & Kik (2000). Subsequently De Melo (2003) studied the genetic basis of the root system of *A. fistulosum* and concluded that it is relatively easy to improve the root system of onion through breeding. Another and complementary approach is the use of arbuscular mycorrhizal fungi (AMF). From earlier studies, it is known that onion plants can

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<sup>1</sup>Plant Research International, WUR, Wageningen, The Netherlands, email: [olga.scholten@wur.nl](mailto:olga.scholten@wur.nl)

<sup>2</sup>University of the Republic, Montevideo, Uruguay

<sup>3</sup>Soil Quality Group, WUR, Wageningen, The Netherlands

<sup>4</sup>Centre for Genetic Resources, the Netherlands (CGN), WUR, Wageningen, The Netherlands

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associate with AMF (Stribley, 1990; Charron et al., 2001). For example, the application of AMF in greenhouse experiments using organically managed soils resulted in yield increases of *Allium fistulosum* between 50 and 60% and significant increase of soil rooting area (De Melo, 2003).

The aim of the present research was to study the beneficial effect of arbuscular mycorrhizal fungi on the growth and development of *Allium* species, and to determine whether it is possible to improve onions for mycorrhizal responsiveness by means of breeding.

### Materials and methods

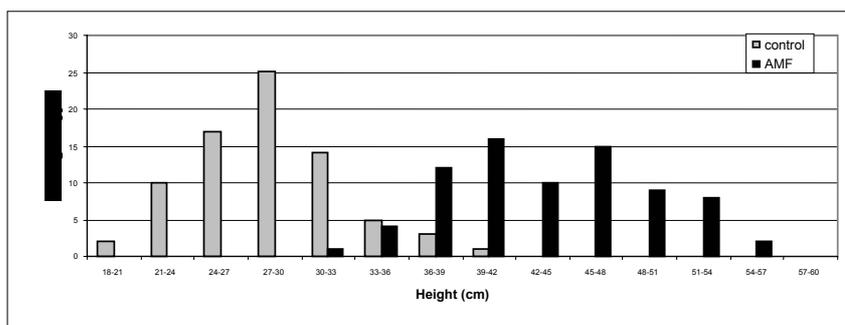
A tri-hybrid population was developed as described by Khrustaleva and Kik (1998). First, *Allium roylei* (RR) was crossed to *A. fistulosum* (FF). A specific RF genotype was chosen as pollen donor in a cross with onion (CC). Consequently, a population of *A. cepa* x (*A. roylei* x *A. fistulosum*) was built (referred to as CCxRF), each genotype carrying a set of *A. cepa* chromosomes and a set of an *A. roylei* - *A. fistulosum* combination. Seventy-seven genotypes were tested for responsiveness. AMF species *G. intraradices*, was kindly provided by Dr. Y. Kapulnik, Volcani Centre Israel.

The experiment was carried out in a climate controlled greenhouse (day/night 22/17 °C), using the population, the parental species and the RF-hybrid. Each genotype was vegetatively multiplied, and transferred to individual pots containing a mixture of sterilized clay soil, sand and perlite (6:1:1, v/v/v). AMF was added to the plant hole just before transplanting. Per genotype, six replications were used with AMF (treated plants) and six with sterilized AMF (control plants, NM). After five weeks, AMF-colonization was quantified using the grid method (Brundrett *et al.* 1996). Colonization ranged from 30-40% in the AMF treatment, and no mycorrhiza was observed in roots of control plants. Plants were harvested thirteen weeks after transplantation. During their growth and also at harvest several characteristics of the plants were measured, such as: total fresh and dry weight, and their partitioning in leaves, bulb or stem, and roots. Also the number of leaves, stems, and roots was recorded, as well as plant height. AMF responsiveness was calculated as the increase in plant height or weight compared to the non-mycorrhiza treatment:  $(W_{AMF} - W_{NM})/W_{NM} * 100$ . Responsiveness was considered significant when the AMF and control treatment were statistically different ( $p < 0.05$ ). In this paper only results of plant height and fresh weight will be given attention. Other results still have to be analysed.

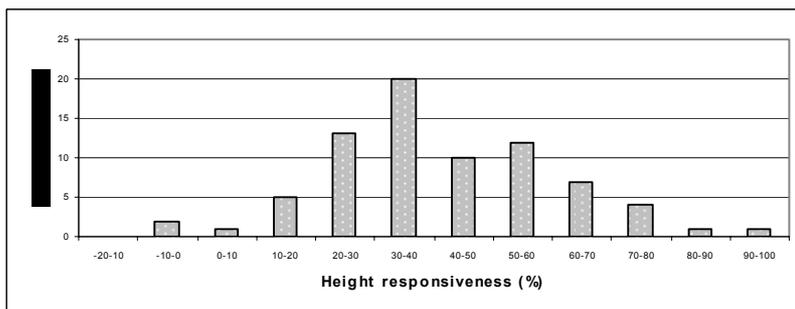
### Results and Discussion

AMF had a significant effect on plant height of the tri-hybrid population. In the control plants the average height of the longest leaves varied between 21 and 45 cm, whereas this varied between 33 and 57 cm when AMF was applied (Figure 1). The frequency distribution of the individual genotypes of the tri-hybrid population for their responsiveness to AMF analysing plant height as the trait of interest clearly demonstrated genetic variation in responsiveness varying from plants that did not or hardly respond to AMF to plants that showed an increase of 100 % in plant height (Figure 2).

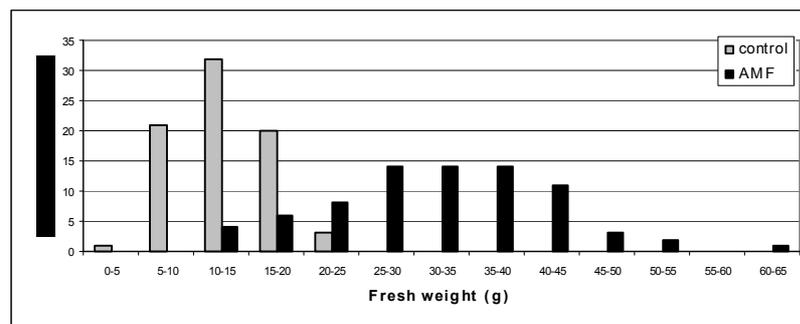
AMF also had a positively influence on total fresh weight of plants at harvest (Figure 3). Treatments with AMF had an average fresh weight between 10 and 65 g, whereas control plants weighted between 0 and 25 g. The CCxRF genotypes segregated from no or hardly any responsiveness to 500% increase.



**Figure 1.** Frequency distribution of individual genotypes of the CCxRF population in plant height classes (longest leaf from the ground level, in cm) for the *Glomus intraradices* treatment (AMF) and the control, nine weeks after inoculation. The distributions do not include the parents.



**Figure 2.** Frequency distribution of individual genotypes of the CCxRF population in plant height responsiveness to *Glomus intraradices* (see text for calculation), nine weeks after inoculation. The distribution does not include the parents.



**Figure 3.** Frequency distribution of individual genotypes of the CCxRF population in fresh weight for the *Glomus intraradices* treatment (+AMF) and the control. The distributions do not include the parents.

Based on the aforementioned results the next step in our research will be the analysis of the genetic basis of mycorrhizal responsiveness in the CCxRF population via QTL mapping. Clarification of the genetic basis may help to find exiting possibilities for the development of onion cultivars more suited for low input farming. The reason for this is that we expect to find not only traits to improve the rooting system but also to improve the mycorrhizal responsiveness by making crosses between *A. fistulosum* and onion.

### Conclusions

The results support the hypothesis that exploitation of *A. fistulosum* is an interesting option to improve onions by breeding to obtain cultivars better adapted to low input farming because of their improved rooting system and mycorrhizal responsiveness.

### Acknowledgments

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