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Specificity and Functional Compatibility of VA Mycorrhizal Endophytes in Association with *Bradyrhizobium* Strains in *Cicer arietinum*

J.M. RUIZ-LOZANO and R. AZCON
Departamento de Microbiología, Estación Experimental del Zaidín CSIC
Prof. Albareda 1, 18008-Granada, Spain
Tel. (958) 121011, Fax (958) 129600

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Abstract

Two vesicular-arbuscular mycorrhizal (VAM) fungi, *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe or *G. fasciculatum* (Thaxter sensu Gerd.) Gerd. and Trappe, were tested for their functional compatibility with two *Bradyrhizobium* strains (Br 185 and Br 192) in *Cicer arietinum*. Observed effects on plant growth and nutrition by dual combinations of *Bradyrhizobium* and VAM fungi ranged from compatible interactions in the case of Br 185 to a pronounced functional incompatibility with stain Br 192. The association of *G. fasciculatum* plus Br 185 resulted in a 30% increase of growth and of nutrient content 24% (N), 42% (P), 32% (K). However, the combination of *G. fasciculatum* plus Br 192 reduced dry weight by (58%) and nutrient acquisition by 31% (P), 54% (K), 65% (Ca) and 44% (Mg). Nodule number also increased (53%) in the case of the compatible interaction and decreased (86%) in the incompatible associations. *G. fasciculatum* was more infective than *G. mosseae* in association with *Cicer arietinum*. The most nodulating *Bradyrhizobium* strain was Br 192. Mycorrhizal and nodular development was reduced in plants inoculated with the most invasive endophytes (*G. fasciculatum* and *Bradyrhizobium* 192). Specific mechanisms such as the reduced level of chlorophyll content conferring functional differences in plants nodulated with the strain 192 of *Bradyrhizobium* could be related. In the present study the specific interaction between endophytes was tested at physiological and structural levels. Plant response depends on the particular combination of *Rhizobium* strain and *Glomus* isolate.

Keywords: functional compatibility, VA mycorrhiza, *Bradyrhizobium*, endophyte

1. Introduction

Tripartite symbioses involving *Rhizobium* spp., a vesicular-arbuscular mycorrhizal (VAM) fungus and a leguminous plant have been the subject of intensive research (Barea and Azcón-Aguilar, 1983). Most information on this topic has focused on the positive interactions found between endophytes, usually measured in terms of growth and nutrition of the host plant. Positive stimulation often resulted from the enhancement of formation and function of symbiotic structures. Nevertheless, reported experiments show that it is not possible to generalize on interactions between symbionts since each particular partner involved needs a specific study (Azcón et al., 1991). Physiological interactions between symbionts and plants form the basis for a functional compatibility between organisms. Since physiological and biochemical processes must be successfully shared in the triple association and root carbohydrates are demanded for the symbionts, the functional compatibility is a careful balanced system that efficiently improves plant growth and nutrition.

Cicer arietinum is one of the important grain legumes in the semi-arid and arid tropics, with over 11 million ha. under cultivation (Auckland and Var der Maesen, 1980). One must emphasize the involvement of the VAM-*Bradyrhizobium* association in contributing to chickpeas nutrient acquisition. Enhanced plant growth and nutrition in the tripartite symbioses is related to the degree of the intersymbionts compatibility. The present study attempts to select for the most appropriate combination of endophytes. We document variations in the responses of *Cicer* to inoculations with different species of symbionts (two *Glomus* sp. and two *Bradyrhizobium* strains). It was examined if the timing of *Bradyrhizobium* inoculation at either sowing time or reinoculation 15 days after sowing, influenced the effects of microbial inoculations evaluating plant growth, nutrient assimilation and symbiotic structures formation. Compatibility is expressed as plant growth and nutrition as well as the development of symbiotic structures. Chlorophyll content of plants nodulated with *Bradyrhizobium* are given as an indication for the physiological plant status.

2. Materials and Methods

Experimental design

The experiments consisted of two single inoculation treatments with *Bradyrhizobium* strain 185 or strain 192 and 8 dually inoculated treatments including combinations of each one of the *Bradyrhizobium* strains plus the VAM fungus *G. mosseae* or *G. fasciculatum*. In half of these simultaneous inoculation

treatments, the *Bradyrhizobium* strains were reinoculated after 15 days. Five replications for each treatment were performed.

Soil and biological materials

The soil collected from Granada province (Spain) with a pH of 8.1, contained 6.24 mg g^{-1} of available P, 0.25 mg g^{-1} of N, 132.00 mg $g^{"-1}$K and 1.81% of organic matter, was sieved (2 mm), diluted with quartz-sand (1/1, soil/sand, v/v) and sterilized by steaming (100° C for 1 hr during 3 consecutive days).

Pots containing 1,000 g were filled with sterilized soil/sand mixture. Mycorrhizal inoculation was with a stock culture of *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe or *Glomus fasciculatum* consisting of soil-containing spores, mycelium and infected root fragments from an *Allium cepa* stock inoculum. The inoculum was added (5 g pot^{-1}) at sowing time, just below the surface of sterilized seeds of *Cicer arietinum*. *Bradyrhizobium* strains from Icarda (Syria) were grown in a rotary shake culture at 28° C for 7 days in sterilized 250 ml flasks containing 75 mL of Allen 79 (1957) medium. The *Bradyrhizobium* inoculum consisted of 2 mL of medium containing 10^9 cells/ mL^{-1}. Inoculum was added on the seeds at sowing time and 15 days after sowing.

Growth conditions

Plants were cultivated in a greenhouse with 16/8 hr day/night cycle and 80% relative humidity. Day and night temperatures varied from day to day, but day temperatures did not exceed 35° C and night temperatures did not fall below 21° C during the experiment. Water was supplied daily to maintain soil moisture close to 80% field capacity during the period of plant growth. At sowing time and two weeks after sowing, water was added to reach an optimum level for seed germination. Plants received weekly 10 mL of Hewitt (1952) nutrient solution lacking N and P.

Determinations

At harvest (8 weeks after planting) the root system was separated from the shoot and dry weights were determined after drying for 36 hr at 70° C.

The number of nodules on the main and lateral fresh roots were counted by direct observation using a magnifying glass.

Visual observation of mycorrhizal infection was made by clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactophenol (v/v) according to Phillips and Hayman (1970) and quantification was performed using the Giovannetti and Mosse method (1980).

Total N content of dried, ground plant material was determined by micro-Kjeldahl assay, phosphorus content was measured by the ammonium molybdate method (Olsen and Dean, 1965) and K, Ca, and Mg were quantified by atomic absorption (Lachica et al., 1973). For chlorophyll content determination, 1 g of fresh leaf tissue was homogenized using 10 mL of Tris-HCl 50 mM, pH 7.5 and filtered. Aliquots of the filtrate (0.4 mL) were added with water (0.6 mL) plus 4 mL of acetone. After 10 min of centrifugation at 6.000×g, the chlorophyll concentration was determined colorimetrically at 625 nm. Results were expressed as mg per g fresh shoot weight. The results were statistically evaluated by analysis of variance and DUNCAN's multiple range test.

3. Results

Bradyrhizobium strains affected plant growth, N,K, Mg nutrient content and chlorophyll content of *Cicer arietinum* in different ways (Fig. 1, Table 1). Strain 192 as single inoculation gave the most efficient enhanced yield. The most relevant result, however, is the differential behaviour manifested in the tripartite symbiosis depending on the particular association of endophytes.

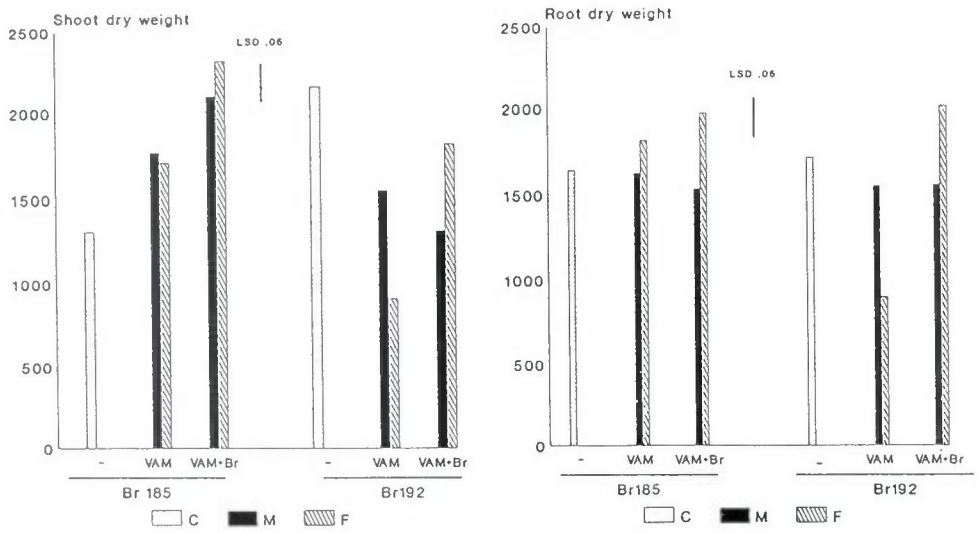

Figure 1. Shoot and root dry weight (mg plant^{-1}) of *Cicer arietinum* plants singly inoculated with *Bradyrhizobium* (185 or 192 strains) (C) or in dual combination with VAM endophytes (*G. mosseae*, M or *G. fasciculatum*, F) inoculated with *Bradyrhizobium* spp. or reinoculated after 15 days (+Br).

Table 1. Nodule numbers, VA mycorrhizal colonization (%) and chlorophyll content in plants of *Cicer arietinum* singly inoculated with *Bradyrhizobium* (185 or 192 strains) in dual combination with VAM endophytes (*G. mosseae*, M or *G. fasciculatum*, F) or reinoculated with *Bradyrhizobium* spp. (2 Br)

Treatments	Nodule number	% VAM colonization	Chlorophyll (mg/g f.w.)
Br185	16.1e	–	7.3a
Br185 + M	23.2cd	10.2c	8.9a
Br185 + F	29.5bc	37.4b	8.6a
2Br185 + M	19.2de	7.5c	6.7a
2Br185 + F	28.1bc	51.4a	7.4a
Br192	35.0b	–	2.2b
Br192 + M	14.9e	3.9c	2.6b
Br192 + F	4.5f	11.7c	2.7b
2Br192 + M	15.0e	6.8c	2.4b
2Br192 + F	45.5a	42.3a	3.3b

Means (5 replicates) not followed by a common letter differ significantly (P> 0.05) from each other (Duncan's multiple range test).

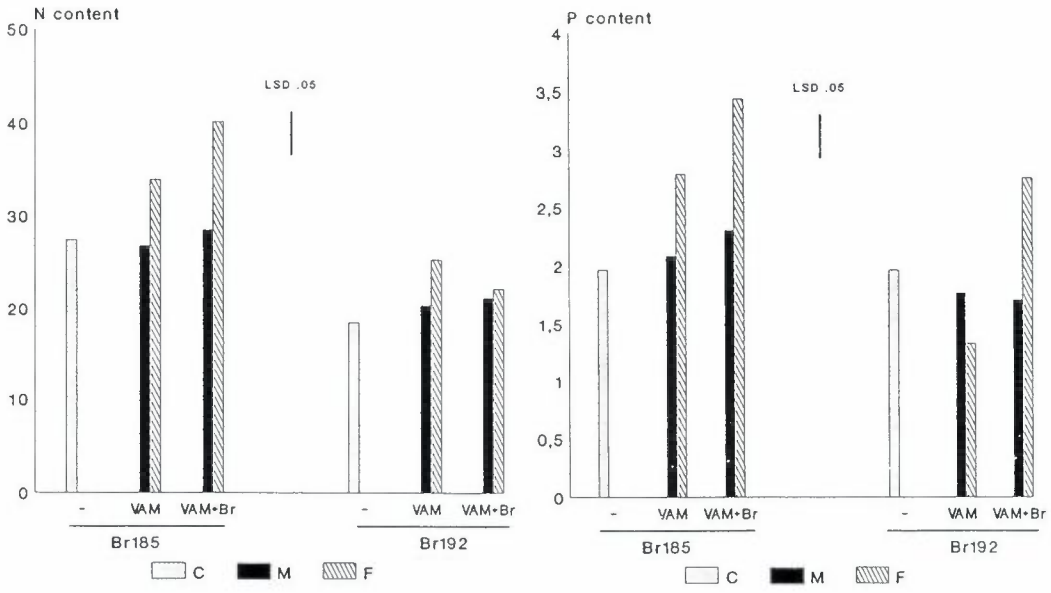

Figure 2. N and P shoot content (mg plant^{-1}) in plants of *Cicer arietinum* singly inoculated with *Bradyrhizobium* (185 or 192 strains) (C) or in dual combination with VAM endophyts (*G. mosseae*, M or *G. fasciculatum*, F) inoculated with *Bradyrhizobium* spp. or reinoculated 15 days after sowing (+Br).

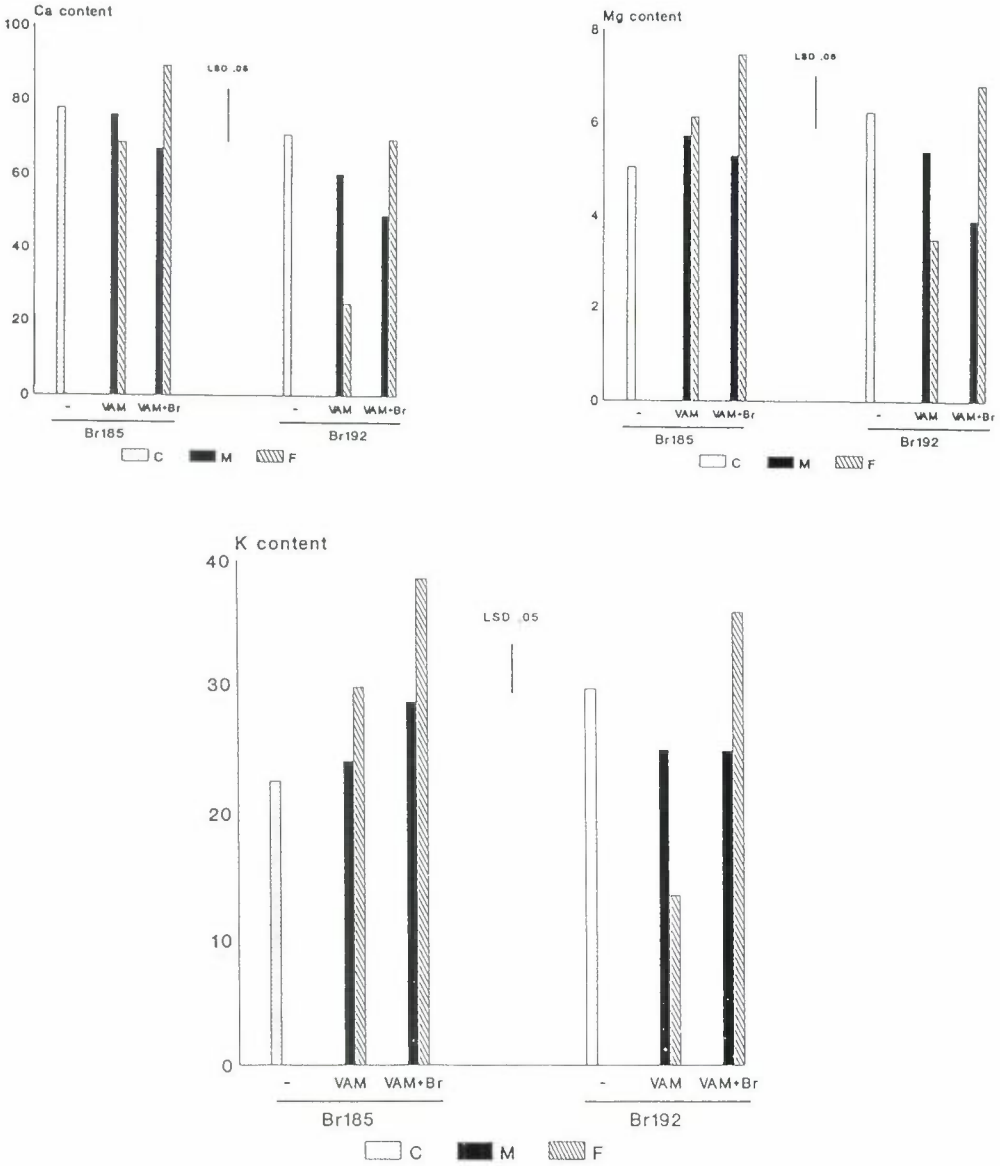

Figure 3. K, Ca and Mg shoot content (mg plant^{-1}) of *Cicer arietinum* plants singly inoculated with *Bradyrhizobium* (185 or 192 strains) or in dual combination with VAM endophytes (*G. mosseae*, M or *G. fasciculatum*, F) inoculated with *Bradyrhizobium* spp. or reinoculated after 15 days (+Br).

In fact, a detrimental effect was found with the dual inoculation of Br 192 plus any mycorrhizal fungus, particularly with *G. fasciculatum*. Such negative interactions greatly decreased the formation of nodules (50% in *G. mosseae*-colonized plants and 86% in *G. fasciculatum*-colonized plants) as well as plant shoot and root biomass.

Conversely, strain Br 185 positively interacted with both endomycorrhizal fungi, enhancing plant dry weight, nodule formation and consequently nutrient content. The positive effect on N, P, and K plant content was greater with the association of Br 185 plus *G. fasciculatum* and resulted in a 30% increase in growth and 24% (N), 42% (P) and 32% (K) increase versus single Br 185 inoculation. However, when Br 192 was the strain associated with *G. fasciculatum*, plant growth decreased by 58% and the nutrient absorption by 31% (P), 54% (K), 65% Ca) and 44% (Mg), respectively versus Br 192 individual inoculation. Nodule number increased by 53% in the case of compatibility and decreased by 86% in the incompatible combinations. Similarly, but with less differences between treatments were observed in root dry weight. Nodule size, shape and colour were similar in all cases.

No relationship seems to exist between percentage of VAM colonization or nodule formation and growth response. Root growth was less affected than shoot growth by microbial treatments, but similar trends were observed (Fig. 1).

G. fasciculatum was the more infective fungus and was the most effective in combination with Br 185 and less with Br 192, the most nodulating *Bradyrhizobium* strain. Mycorrhizal and nodular development was reduced in the plants inoculated with the most infective endophytes (*G. fasciculatum* and Br 192).

When an initial simultaneous inoculation of endophytes was followed with a second reinoculation of *Bradyrhizobium*, no differences in the general results previously obtained were observed: effective dual VAM inoculation with Br 185 and ineffective with Br 192. Nevertheless, *G. fasciculatum*-colonized plants were always favoured in growth, nutrition and symbiotic developments (nodules and VAM infection) by the reinoculation of *Bradyrhizobium*.

Values for leaf chlorophyll content showed significant differences according to the *Bradyrhizobium* strains inoculated. Curiously, the better nodulating Br 192 strain considerably decreased chlorophyll content when compared with Br 185 inoculated plants. No variations on this parameter between treatments were found in plants colonized by each *Bradyrhizobium* strain.

4. Discussion

The effectiveness of dual inoculations involving VAM-fungal species and *Bradyrhizobium* strains depends on specific combinations of endophytes. Results here show that strain 185 positively interacted with both *Glomus* spp. while interaction of strain 192 of *Bradyrhizobium* and VAM endophytes was highly negative. The importance of an appropriate selection of VAM fungal species in a system including *Rhizobium* strains was suggested by recent studies (Ianson and Linderman, 1989; Von Alten et al., 1989; Azcón et al., 1991). The effectiveness found in the combination between one of three *Glomus* spp. and one of 6 *Rhizobium meliloti* strains depended on the degree of intersymbiont compatibility (Azcón et al., 1991), but all the combinations resulted in either a positive effect or had no effect on host growth. In the present study, a stronger effect between specific combinations of strains and species of associated endophytes was seen. The interaction between the symbionts ranges from compatible (Br 185 plus VAM-fungus) to incompatible (Br 192 plus VAM fungus), with respect to plant growth and nutrient uptake.

Since in symbiotic relationships, host root carbohydrates are required by the microsymbionts as nutrition and energy sources, C limitations may affect endophyte development (Bethlenfalvay and Pacovsky, 1983). *Bradyrhizobium* strains differentially affected chlorophyll content in *Cicer arietinum*. This parameter can be taken as an indirect indication of photosynthesis (Nemec and Vu, 1990), and it was highly reduced by the better nodulating strain Br 192. This fact could explain the negative effect that *Glomus* sp. had on plant nodulated with Br 192. Kucey and Paul (1982) estimated that 10% of root carbohydrates were consumed by the metabolic activity of VAM fungi in colonized roots.

The benefit that a legume receives by possessing mycorrhizae depends upon the physiological differences of the fungus associated in connection with this, the most infective fungus, *G. fasciculatum*, was the most incompatible endophyte in association with Br 192. Kucey and Paul (1982) suggested that *G. fasciculatum* has a higher C requirement or displays less photosynthetic compensation than *G. mosseae*. Herold (1980) made the cost/benefit evaluation in the symbiotic system in terms of photoassimilates and in this way the results can be regarded as symbiotic interactions. Functional compatibility in the mutualistic association is defined as a balanced physiological interaction which contributed to the fitness of partners. Differences in the sensitivity to their photosynthetic and nutritive interchanging mechanisms between partners could be a possible basis for specific responses in these tripartite symbiotic systems.

Brown and Bethlenfalvay (1986) pointed out that individual inoculation reached better levels of infectivity. Our results show that no generalization on colonization interference between endophytes can be made since it depends on particular combinations of strains, species or isolates of endophytes involved.

Evidence supporting the lack of competition between endophytes at colonization sites was tested with the reinoculation of strain 192 of *Bradyrhizobium* in the incompatible association which increased both symbiotic developments (nodules and VAM infection). On the basis of this result, no relationship between symbiotic formation and function was evidenced since percentage of VAM colonization or nodule formation seem not to be related with growth response.

Regulation of the symbiosis involves a combination of mechanisms that either limit or promote infection, depending upon environmental conditions. When photosynthesis is limited, the rate at which VAM infection is established are often reduced. Low values of leaf chlorophyll content in plants nodulated by Br 192 strain may account for endophyte activity. In this sense, Son and Smith (1988) pointed out that light limitation did not reduce VAM root colonization, but efficiency on plant growth was affected. The endophyte-host interaction in the present mutualistic symbiosis was influenced at physiological or functional, rather than at a structural level. However, with the available data, it is difficult to deduce the causes accounting for the incompatibility tested between specific couples of endophytes.

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