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CHARACTERIZATION OF ITALIAN CHICKPEA LANDRACES AND INVESTIGATION ON INTERACTION BETWEEN PLANT, PATHOGEN AND BIOCONTROL AGENTS

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Abstract 4
1. Introduction 6
1.1 Botany 6
1.2 Plant Cultivation 7
1.3 Main pathogens 9
1.4 *Fusarium oxysporum* f.sp. *ciceris* 9
1.5 *Ascochyta rabiei* 10
1.6 Aims of the research 11
2. Materials and Methods 12
2.1 Landraces characterization 12
2.1.1 Controlled condition pathogenicity test to evaluate the resistance of chickpea landraces to *Fusarium oxysporum* f. sp *ciceris* 12
2.1.2 Field trials to evaluate the resistance of chickpea landraces to *Fusarium oxysporum* f. sp. *ciceris* 13
2.1.3 Controlled condition pathogenicity test to evaluate the resistance of chickpea landraces to *Fusarium oxysporum* f. sp *ciceris* race 5 19
2.1.3 Field trials to evaluate the resistance of chickpea landraces to *Ascochyta rabiei* 13
2.1.3 Field trials to evaluate the resistance of chickpea landraces to *Fusarium oxysporum* f. sp. *ciceris* race 5 19
2.1.4 Agronomics traits 14
2.1.5 Genotypic characterization of landraces 14
2.1.6 Data assessment and statistical analysis 15
***2.2* Plant, pathogen and BCA interaction** 16
2.2.1 Effect of BCAs on different chickpea genotypes 16
2.2.2 Pathogenic mechanisms of *Fusarium oxysporum* f.sp. *ciceris* race 0 17
3. Results 19
3.1 Landraces characterization 19
3.1.3 Field trials to evaluate the resistance of chickpea landraces to *Ascochyta rabiei* 20
3.1.4 Agronomic traits 20
3.1.5 Genotypic characterization 21
3.2 Plant – pathogen – BCA interactions 22
3.2.1 Effect of BCAs on different genotypes 22
3.2.2 Pathogenic mechanism of *Fusarium oxysporum* f.sp. *ciceris* race 0 23
4. Discussions and Conclusions 24
5. Tables 27
6.Figures 30
7. Bibliography 44

Abstract

Chickpea is the third pulse crop in the world and for its nutritional features represents an optimal source of nutrients, vitamins and minerals in both human and animal diet. Italy is the second chickpea producer in the European Community (EU), but the Italian production, all destined for human consumption, is mainly based on local landraces growing in little farms. From 1985, because of a sudden decrease of the chickpea national production, most part of Italian market is supported by imports. This trend, fortunately, is reversing because of the attention that consumer deserve to local products and consequently the growers are stimulated to reintroduce the chickpea in the crop rotation schedules. Furthermore, studies supporting the beneficial effect of a chickpea integrated diet on human health also encourage the consumer to use this legume in the diet more than in the past. On the other hand, farmers are often hesitant in introducing this crop in their cultivation plan because of yield casualty due to adverse agronomical factors as well as some severe plant diseases such as those caused by *Fusarium oxysporum* and *Ascochyta rabiei* that could limit the use of some local landraces.

For a sustainable production, it is important to integrate traditional control strategies, such as genetic resistance, with use of Biocontrol Agents (BCAs). By this work eighteen Italian landraces where characterized for some important agronomic traits and for their genetic resistance to the above mentioned fungal pathogens.

Another investigated aspect was the influence on disease development of BCAs combination with the landraces genetic resistance. To this aim, a pair of Near Isogenic Lines (NILs) of chickpea, carrying a major gene of resistance to *Fusarium oxysporum* f. sp. *ciceris* race 0 (*Foc*0) were infected with the pathogen and treated with some BCAs. The infective mechanisms of this pathogen were also investigated supplying the NILs plants with solutions at different concentration of EDTA or fusaric acid.

Results showed the presence of genetic resistance to *Foc*5 in some landraces used together with other useful and appreciable traits. Other landraces show different degrees of resistance to *Ascochyta rabiei* too. Despite the considerable degree of genetic variability evidenced by genetic analysis, the examined landraces could be used as starting material in breeding programs.

Experiments conducted on NILs gave encouraging results on the potential use of integrated strategy based on the combination of genetic resistance with BCAs. These experiments also represent an advancement in the understanding of the infective mechanism of *Foc*0.

1. Introduction

Cickpea (*Cicer arietinum* L.) is the only cultivated species of the genus *Cicer*. It belongs to the tribe *Cicereae,* subfamily *Papilionoideae,* family *Fabaceae.* Latin name of chickpea, *Cicer*, derived from the Greek *kikus*, which means strength. Other hypothesis is that it came from the Hebrew *kirkes*, where *kikar* means round (Duschak 1871). Another Greek name of chickpea is *krios*, which also means ram (*aries* in Latin), in fact, the word *arietinum* in its binary nomenclature is a clear reference to the shape of the seeds, which remember the head of a ram.

1.1 Botany

The chickpea origin is from East Turkey were its ancestor *Cicer reticulatum* Ladiz was discovered (van der Maesen 1987). The lineage of chickpea from this species was proved by induced mutations in *Cicer reticulatum*, which produced mutants with traits similar to *Cicer arietinum (Toker 2009)*.

Cultivated chickpea is divided in two distinct type: desi and kabuli (Fig.1). The desi type is also called microsperma because of the small size of seeds (0.1 – 0.3 g per seed), with colored and thick coat and rough surface; this type is characterized by pink flowers and anthocyanin pigmentation of stems, it is largely cultivated in Eastern Africa and some areas of Nile Valley and Asia. kabuli types, also called macrosperma (0.2 – 0.6 g per seed), take its name from the city of Kabul (Afghanistan), where it is supposed to be selected in the past. Kabuli seeds are white or beige colored, characterized by a ram's head shape, with a thin coat and smooth surface. The flowers are white and stems green for lacking anthocyanin (Moreno and Cubero 1978).

Seeds are constituted by two cotyledons and an embryo enveloped in a coat formed by two layer, *testa* and *tegmen* (outer and inner layer respectively), and a *hilum*, which is the point of attachment to the pod. Embryo consists of an axis and two fleshy cotyledons. The pointed end of the axis is the radicle and the feathery end the *plumule*. Best germination is triggered by optimum of temperature (28 – 33°C) and high soil moisture for 5-6 days. It begins with the swelling of the seeds by absorption of moisture, followed by emerging of radicles and then of the *plumule*. Elongation of epicotyl push the *plumule* upward; *plumule* growing will produce an erect shoot and with the first true leaf having two or three pairs of leaflets plus a terminal one, the plumular shoot and lateral branches grow continuously to develop into a plant. Stem could have a grow habit from prostrate to erect, it is herbaceous and hairy. From the ground level plant will develop primary branches, and from these, the secondary and tertiary branches.

Leaves are petiolate, compound uniimparipinnate. The rachis is 3 – 7 cm long and supports 10 – 15 leaflets. The whole plant, except for the corolla, is covered by glandular hairs with defending function by releasing of oxalic acid. Radicle will produce the roots characterized by a strong taproot with three or four rows of lateral roots. Roots system could reach 2 m of length and its parenchymatous tissues is reach in starch; root system bears *Rhizobium* nodules for nitrogen fixation (Singh and Diwakar 1995).

Chickpea flowers are solitary, but some mutants will have 2 or 3 flowers per node (Rubio et al. 2004). The flower has five petals (*vexillum*, wings and keel), there are 10 stamen in diadelphous condition with filaments of 9 stamen fused and the 10^{th} free. The ovary is monocarpellary, unilocular and superior with marginal placentation, in it there are 1 – 3 ovules. Fecundation occurs when flower is hooded and anther dehiscence take place 1 day before the opening of the flower, so the plant is completely autogamous (cleistogamy). Pod formation begins 5 – 6 days after fertilization, it will carry 1 – 2 seeds rarely 3 (Singh and Diwakar 1995).

1.2 Plant cultivation

Chickpea plant has a poor nutrient's requirement and represents an important tool in crops rotations to restore the soil fertility. Since symbiotic legume plants, including chickpea too, don't need nitrogen fertilizations, but are very sensitive to phosphorous and microelements deficiency, especially, in alkaline soils as well as to saline stress. Chickpea is cultivated in every kind of soil, but it prefers loamy or clay-silty soils with a good degree of aeration and a range of pH between 5.7 and 7.2 (Singh and Diwakar 1995).

Chickpea resists to drought stress (it requires between 110 and 280 mm of water per years) and its nutrients composition make of it a good cultivation for arid and semi-arid area (Rossini 2008).

The 80% of chickpea dry seed mass is composed by proteins and carbohydrates (especially Dietary fibre), the last ones are more present in chickpea than in other pulses. Chickpea is also a good source of phytosterols, vitamins and minerals, thus its grain represent a good element in association with cereals for diet in poor areas of the world. Considering benefits conferred by a chickpea integrate diet on disease like diabetes, obesity, cancer, blood pressure and other diseases of the cardiac system, chickpea represent also a nutraceutical resource for rich countries (Jukanti et al. 2012).

The traditional cultivations areas of chickpea are the Mediterranean basin including middle eastern countries (kabuli type) and the Indian subcontinent (cultivation of desi type chickpea). Its cultivation spread to the Americas and Australia (kabuli type) and to the eastern Africa

where is subjected to the altitude. The cultivation system and the type of chickpea cultivated is strongly linked with the area of cultivation. In fact, In the Indian subcontinent, where the desi type is the main cultivated, chickpea follows oleaginous plant, while in the Mediterranean basin, where is cultivated mainly kabuli type (except for the Nile valley), chickpea is used in rotation with grains and fruit or vegetable crops (grains and other legumes in Nile valley) in other areas cultivation is followed by grains or by another cycle of chickpea (Singh and Diwakar 1995).

Chickpea represents, actually, the third pulse produced in the world after soybean and bean and in Europe the major producer country is Spain, followed by Italy, Portugal and Balcanian Countries (FAOSTAT, 2014).

In Italy chickpea is the third cultivated legume with a surface of 9037 ha and a production of 13072 t (FAOSTAT, 2014). Due to the high fragmentation of Italian farms (ISTAT, 2015) only some big farms grow selected varieties of chickpea. Thus, the crop is mainly carried out by little farms which make a large use of landraces, generally appreciated on local market as typical product. These local ecotypes usually take their name from the locality where they are traditionally cultivated (Negri 2003).

Although Italy is the second European producer of chickpea, its cultivation is still limited by the fortuity of the yields due to different adverse environmental and/or agronomical factors (Rossini 2008).

Actually, in Italy, the production as the import of chickpea are only intended for human consumption, while its introduction in animal feed could represent a viable alternative to soybean, becoming a valid turnover in crop rotations, especially in the dry arid climates of Southern Italy (Crinò and Saccardo 2008).

Using landraces is important not only by an economic point of view; landraces could also play a social and cultural role in agriculture. In fact, he use of landraces, sometimes, is connected to ethnic characteristic of linguistic minority, especially in Central and Southern Italy, where many programs of cultivated plant biodiversity preservation are carried out to constitute germplasm's banks aimed at the storage of this ecotypes (Laghetti et al. 2011).

To make profitable the crop, it is necessary to increase chickpea yield by exploiting the full cycle of the plant sowing the crop in winter. Actually winter seeding is strongly limited by climatic factors which affect germinability, young plant's viability, diseases and the high appearance of weeds (Rossini 2008).

1.3 Main pathogens

Chickpea's cultivation could be affected by a wide range of pathogens such as *Rhizoctonia bataticola* causing dry root rot, *Fusarium solani* f.sp *pisi* causing the stem rot (Nene et al. 1981) and emerging pathogens such as *Fusarium oxysporum* f sp. *pisi* (De Curtis et al. 2014).

The economically most important diseases of chickpea are ascochyta blight, caused by *Ascochyta rabiei*, responsible for losses calculated in about 542 million US$, fusarium wilt caused by *Fusarium oxysporum* f. sp *ciceris* and responsible for losses estimated in 260 million US$ and grey mold caused by *Botrytis cinerea* with 92 million US$ losses estimated (VV. 2000).

1.4 *Fusarium oxysporum* f.sp. *ciceris*

The genus *Fusarium* consists of a wide range of plant pathogenic fungi with a large variety of hosts and infection strategies characterized by spindle shaped macroconidia. Among this, *Fusarium oxysporum* differs in several ways: first, its apparent long history of asexual reproduction; its infection mechanism; its gene – for – gene relationships with several host which causes the presence of different host specific forms (*formae specialis)*. *Fusarium oxysporum* is a soil borne fungi which invades roots tissues and can cause wilt diseases through colonization of xylem. Due to its exclusive asexual reproduction, it is regarded as a species complex and each *formae specialis* consist of one or several clonal lines corresponding to vegetative compatibility groups (Michielse and Rep 2009). A clear example is the *Fusarium oxysporum f. sp. ciceris* (*Foc*), the causing agent of Fusarium wilt in chickpea. Actually, eight races of *Foc* are described; each one bases on differential specific varietal response and geographical distribution. The eight races are grouped in two pathotype basing on the symptoms: the races 0 and 1 B/C (Mediterranean basin and California) belong to the pathotype yellowing (Fig. 2) which sometimes cause only xylematic lesions (Fig. 3); the races 1A, 5, 6 (worldwide-distributed), 2, 3 and 4 (Indian subcontinent) belong to the pathotype wilting (Fig. 4) (Nogales Moncada et al. 2009).

The most important metabolic difference between the two pathotypes consist in the production of fusaric acid, present in the yellowing races and absent in the others (Palmieri 2016).

del Mar Jiménez-Gasco et al. (2004) propose two evolution models for the 8 races based on the presence of three (model one) or one (model two) nonpathogenic *Fusarium oxysporum* ancestors. The development of disease is largely influenced by several variables such as the inoculum density, the sowing date and the genetic compatibility with the cultivars of chickpea. In fact, springer sowing crops are often more affected by the disease because seedling will

encounter the pathogen in a more susceptible stage (Navas-Cortés et al. 1998). At the same time, sowing for several years the same cultivar in the same parcel will increase the severity of the disease. Then, if not appropriately managed, fusarium wilt due to *Foc* will cause several economic damages. This occurs because *Foc* produce clamidospore by which will stay in the soil for more than six years (Arunodhayam et al. 2014).

Actually, the management of Fusarium wilt disease consist in very long crop rotation and the use of resistant varieties. The persistence of *Fusarium* conidia in the soil leads to crop rotation not shorter than five years (Sharma and Muehlbauer 2007). Although resistance commercial varieties are available, chickpeas fusariosis is peculiar to local varieties and ecotypes, but genome mapping can be a valuable tool for genetic improvement. From the first steps on mapping the chickpeas genome (Winter et al. 2000), a wide range of molecular markers associated to the genetic resistance to different races of *Foc* are available. So we know that there is a gene cluster conferring resistance to Races 0, 1, 2, 3, 4 and 5 (FOC5, dominant an conferring resistance, foc5, recessive and conferring susceptibility) on chromosome 2 (Sharma and Muehlbauer 2007), we know a second gene ($FOC0_1/foc0_1$) conferring resistance to the race 0 located on chromosome 5 (Rubio et al. 2003; Jendoubi et al. 2016). Even if we don't know which genes are directly involved in genetic resistance, we have the molecular tools for a correct chickpea's breeding (Millan et al. 2010).

1.5 *Ascochyta rabiei*

Ascochyta rabiei (teleomorph *Didymella rabiei*) is the causal agent of ascochyta blight in chickpea, it is an air borne fungi which infect leaf, stem and flower (Fig. 5) and pod (Fig. 6). The anamorph will infect the crops and is characterized by formation of pycnidia, while the teleomorph, when present, will over -winter on crop's debris producing pseudothecia containing cylindrical ascospores. The presence teleomorph will occurs only when the two mating type(MAT1-1 and MAT1-2) are present in the same geographic area (Pande et al. 2005). Pathogen is normally introduced by airborne inoculum or using of infected seeds. In fact, the pathogen is able to grow from the pod wall into the seed when it is still immature and could be found in seed coat or in the cotyledons. At temperatures ranging from 10 to 35°C *A. rabiei* can survive on infected debris for at least 8 month, but burying them the survival time is drastically reduced (Shaid et al. 2008). The disease will appear with lesions on the aerial parts of the plant and is promoted by low temperatures and high Relative Humidity, so will affect chickpea especially in winter sowing. In some cases, the severity of disease could reach the 100% of the crops causing very important economic losses (Pande et al. 2005).

Chickpea shows a horizontally resistance to this pathogen determined by a large number of QTls (Iruela et al. 2006; Du et al. 2011; Madrid et al. 2012; Ahmad et al. 2013). However, actually molecular breeding for introduce resistance to the pathogen is advancing (Bouhadida et al. 2013), also because management of disease is very hard, and ascochyta blight is one of the most limiting factor to the use of winter sowing of chickpea, which is a potential to increase the yield of the crop (Rossini 2008).

1.6 Aims of the research

Chickpea is a re-emerging crop in Italian agriculture and represent a potential resource especially for some areas of Southern Italy (Crinò and Saccardo 2008). Actually the cultivation in Italy is limited by the presence of a small number of varieties and the large use of landraces which are more susceptible to diseases (Negri 2003). Also diseases such as ascochyta blight will prevent the use of the entire cycle of the plant by winter sowing, which would increase the yield of the crops (Rossini 2008).

The most damaging pathogen-affecting chickpeas and causing up to 100% of losses are *Fusarium oxysporum* f. sp. *ciceris* and *Ascochyta rabiei* (Pande et al. 2005; Arunodhayam et al. 2014). Therefore, the characterization of some landraces, focusing on the resistance against these two pathogens, represents an important step for breeding programs. Such programs are useful for the utilization and preservation of landraces, which are an important part of the Italian agricultural biodiversity and also would represent an economic resource, especially for the smallest farms (Arunodhayam et al. 2014; Laghetti et al. 2011).

To improve the molecular tools useful for the breeding programs it is important to investigate the mechanisms which modulate the both infection process and disease development, being this knowledge crucial to direct molecular breeding on specific genes as well as to develop new strategies for disease management.

In agricultural systems which are progressively reducing the use of synthetic pesticide, it is very important to developed new controlling strategies, integrating the use of genetic resistance and Biocontrol Agents (BCAs).

2. Materials and Methods

2.1 Landraces characterization

The vegetal material characterized during my research activity consists in 18 chickpea landraces collected by ARSARP (*Agenzia Regionale per lo Sviluppo Agricolo Rurale e della Pesca* – Campobasso, Italy) the landraces are listed in the table 3.
As controls two ICARDA lines (ILC3279 and WR315) and an Andalucian cultivar (*Blanco Lechoso*) were also used. In genotypic analysis forty-eight Spanish cultivars were also used.
For pathogenicity tests in controlled condition a strain of *Fusarium oxysporum* f.sp *ciceris* race 5 (*Foc*5) isolated in Spain, identified and characterize by morphological and molecular methods, by del Mar Jiménez-Gasco et al. (2004) was used. For pathogenicity tests in field condition two natural strains of *Foc*5 and *Ascochyta rabiei* were used.

2.1.1 Controlled condition pathogenicity test to evaluate the resistance of chickpea landraces to *Fusarium oxysporum* f. sp *ciceris*

For Pathogenicity test in controlled conditions the eighteen landraces and the two ICARDA lines, used as control, were sown in rectangular trays (60 x 40 x 10 cm) filled with sterile perlite and in each tray 5 landraces and/or lines were sown. For every landrace or line 10 seeds in a row traced in parallel to the short side of the tray were sown, the distribution of each row in the tray was randomized and for each line two replicate were sown.
When plants reached the third true leaf stage (about two weeks after seedlings emergence), they were infected by the pathogen. For infection, the pathogen was grown in 250 ml of Potato Dextrose Broth at 25°C kept on a rotary shaker at 120 rpm for about a week. After the growing of the pathogen, the broth was filtered by sterile cheesecloth to remove the mycelium and centrifuged at 4000 rpm for 4 minutes at 4°C to collect macro and microconidia. The latter, after their quantification by a hemocytometer were used for artificial infections. Before infection, plants were explanted and submerged in a water suspension of conidia at concentration of 10^6 conidia/ml where the roots were cut. Plants were left in this suspension for 5 minutes before their replanting in the same perlite in which they were previously grown. During the assay, plants were supplied with a nutrient solution containing: $Ca(NO_3)_2*4H_20$ 1.18 g/l; KH_2PO_4 136.0 mg/l; KNO_3 515.5 mg/l; $MgSO_4*7H20$ 492.96 mg/l EDDHA Fe 6% 18.8 mg/l; KCl 3.692 mg/l; H_3BO_3 1.546 mg/l; $MnSO_4*H_20$ 0.338 mg/l $ZnSO_4*7H_2O$ 57.6 mg/l; $CuSO_4*5H_2O$ 0.126 mg/l $Na_2MoO_4*2H_20$ 0.46 mg/l.

For each landrace four replicates (plants) were used and the experiment was repeated two times.

2.1.2 Field trials to evaluate the resistance of chickpea landraces to *Fusarium oxysporum* f. sp. *ciceris*

To evaluate the resistance degree against *Foc*5, eighteen landraces were sown in the soil of two parcels in field naturally contaminated by the pathogen. The pathogen was identified as the race 5 of *Fusarium oxysporum* f.sp. *ciceris* (*Foc*) in previous years by using variety of chickpea with differential responses to the eight races of *Foc*. Quantification of inoculum was assessed by plating samples of soil and was calculated as 2.5 x 10^5 CFU/g of soil.

For each landrace ten seeds were sown at 10 cm of distance each from other, and at a depth of about 10 cm. After seedlings emergences (from 15 days later) the number of appeared seedlings was assessed and up to 3 months later *Foc* symptoms were periodically assessed.

The evaluation of resistance of chickpea landraces to *Foc* were carried out in two different fields and at two different sowing times: in winter (January) landraces were sown in the experimental field of the iFAPA (*Centro Alameda del Obispo*, Cordoba 37°51'32.3"N 4°48'02.5"W) and in spring (March) they were sown in the experimental field of the Cooperative *Campo de Tejada* – Agrovegetal (Escacena del Campo, Huelva – Spain 37°24'00.3"N 6°23'30.8"W) (Escacena del Campo, Huelva – Spain 37°24'00.3"N 6°23'30.8"W).

Foc disease symptoms, after seed emergence, were assessed every 30 days and the disease level was evaluated as the percentage of leaves showing symptoms for each individual plant rated on a scale of 0 to 4, where 0 = no disease symptoms, 1 = 1 to 33% of leaves with symptoms, 2 = 34 to 66 % of leaves with symptoms, 3 = 67 -100 % of leaves with symptoms, 4 = 176 dead or dying plant (Landa et al. 2004).

2.1.3 Field trials to evaluate the resistance of chickpea landraces to *Ascochyta rabiei*

Evaluation of resistance against *Ascochyta rabiei* was carried out in the experimental field of IFAPA by sowing the landraces in January. In three different parcels, 20 seeds per landrace were sown in 2 meters long rows and with landraces distribution randomized in each parcel. After each four rows sown with the tested landraces as well as along the border of each parcel the *Blanco Lechoso* Spanish *chickpea* susceptible variety was inserted. March plant debris of the *Blanco Lechoso* variety infected in the previous year by *A. rabiei* and stored at 4°C were spread in the experimental parcels and then nebulized with water to increase both relative humidity and conidia dispersal in the field

At the first symptoms appearance *A. rabiei* symptoms on landraces were assessed by using the Singh disease scale (Singh and Hawtin 1981).

2.1.4 Agronomics traits

For each landrace, on same plants of pathogenicity test carried out in the IFAPA field, data about the number of emerged plants after 15 days, flowering rate (assessment at 50% full opened flowers) and growth habit, were also collected. The growth habit was assessed by giving to each plant a value from 0 to 3 (0: prostrate, bushy growth; 1: medium erect; 2: not properly erect; 3: erect) and by calculating the average value for each landrace.

2.1.5 Genotypic characterization of landraces

A bulk extraction of DNA was performed on 0.1 g of leaflets collected from all the plants of the 18 landraces used in experiments conducted under controlled conditions (pathogenicity test). Samples of leaflets from 43 Spanish cultivars seeded in growth chamber were also collected. The samples were frozen in liquid nitrogen and the DNA was following extracted by using the Plant DNAzol® Reagent (Invitrogen™) according with the manufacture recommendations, the extraction protocol was the following:

1. In a chemical hood a 2 ml microcentrifuge tube containing the frozen tissues and with perforated caps were putted in a polystyrene box with specific supports for microcentrifuge tubes and filled with liquid nitrogen to avoid the thawing of the tissue;
2. tissue in each tube was grinded by using a microcentrifuge pestle, during this step liquid nitrogen was added to the polystyrene box when needed;
3. when all the samples were grinded, 300 µl of Plant DNAzol® were added to each tube and the mix of tissues plus reagent was transferred in a new tube, which were incubated at 25°C, 110 rpm for 5 minutes in an Eppendorf ThermoMixer
4. after this step 300 µl of chloroform were added to each sample and incubation was repeated as above;
5. after incubation, samples were centrifuged at 12000 rpm for 10 minutes at room temperature and the aqueous phase containing the DNA was transferred in new tubes;
6. each sample was added with 225 µl of 100% of ethanol, tubes were mixed by inverting and then were incubated at room temperature for 5 minutes;
7. DNA was precipitated by centrifugation at 5000 rpm and 4°C for 4 minutes; the supernatant was discarded and the pellet was re-suspended in 750 µl of 75% ethanol

and incubated at room temperature for washing by centrifugation at 5000 rpm and 4°C for 4 minutes;

8. supernatant was discarded and about 50 µl of TE Buffer (pH 8) were added to each tube, then the tubes were incubated at 37°C overnight to allow DNA dissolving.

DNA was then quantified by Nanodrop and diluted in work solution with the work concentration of 25 ng/µl, used in PCR reaction to amplify the microsatellite listed in Table 1 and known to be located in different linkage groups of the chickpea's genome (Winter et al. 2000; Millan et al. 2010). Each landraces were also analysed by using markers associated with genes for resistance to *Foc*5 (Castro et al. 2010) and *Foc*0 (Jendoubi et al. 2016).

Microsatellites alleles were visualized by electrophoresis in agarose (2.5% w/v), and polyacrylamide (10%, C2, 67%) gels or capillary electrophoresis using an automatic capillary sequencer (ABI 3130 Genetic Analyzer Applied Biosystems, Madrid/HITACHI, Madrid) at the Unit of Genomics of the Central Service for Research Support of the University of Córdoba (Spain). Data of the Fragment Analysis were analysed by using the GeneMapper software and the Peak Studio V2.2 software (McCafferty et al. 2012).

2.1.6 Data assessment and statistical analysis

During the pathogenicity tests, data on number of healthy plants and on plant showing light symptoms (yellowing and/or loss of leaf turgidity), heavy symptoms (withering) or dead were periodically assessed. Data were used to draw the Disease Progress Curve after calculating the AUDPC (Area Under the Disease Progress Curve) by the following equation:

$$AUDPC = (Tn - T1) * D1 + \sum_{i=0}^{n} \left(\frac{1}{2} * \Delta Ti * \Delta Di \right)$$

where *T* is the time of evaluation and *D* is the percentage of death plants at the assessment time and *n* is the number of disease assessments.

By using data from controlled conditions pathogenicity tests, the average AUDPC and the standard deviation between the two tests was calculated.

Data about severity of disease in field condition experiments were used to calculate the McKinney index (McK) using the following equation:

$$McK = \frac{c * f}{N * X} * 100$$

where c is the class of severity assigned during the evaluation (from 0 to 4), f is the frequency of each class for each tested genotype, N is the number of plant for each tested genotype, X is

the highest class of the scale used (4 in this case). For statistical validation of data by ANOVA, the McKinney index data were previously converted into angular value using the function:

$$Arcsin\sqrt{\frac{McK}{100}}$$

Replicates of data collected from controlled and field experiments were subjected to ANOVA by using the SPSS software version 21 and means were separated by Tukey test.

The allele frequencies of data obtained in the diversity analysis experiments were calculated and used to determine the statistical parameters and PIC (Polymorphism Information Content) of each marker.

To create a binary data matrix, the alleles were scored as present or absent with the number 1 or 0 respectively, and this matrix was used to calculate the degree of genetic similarity between all pairwise combinations using the Dice Coefficient of Similarity. Clustering of the genotypes was determined by using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Statistical analysis was performed by using the NTSYS-pc 2.02j software.

2.2 Plant, pathogen and BCA interaction

2.2.1 Effect of BCAs on different chickpea genotypes

To investigate the effect of some BCAs isolated from different plant and soil have on the genetic resistance to *Foc* in chickpea two pathogenicity tests in controlled conditions were conducted using two Near Isogenic Lines (NILs) carrying the genes $FOC0_1/foc0_1$ on chromosome 5.

The NILs used were the RIP10-RIL21p14 (P14), carrying the gene $FOC0_1$ (conferring resistance to the pathogen), and the RIP10-RIL21p5 (P5) carrying the gene $foc0_1$ (Jendoubi et al. 2016).

The 2 NILs were seeded (20 seeds for each NIL for tray) in 18 trays (60 x 40 x10 cm) filled with perlite and as control the Cr5, a genotype of *Cicer reticulatum* - the ancestor of *Cicer arietinum* - known to be very susceptible to *Foc*0 was used. When the plants reached the 3rd true leaf stage (about 9 cm of highness) they were infected by cutting the roots during their immersion for 5 minutes in an aqueous suspension of *Foc*0 conidia at the final concentration of 10^6 conidia/ml. Plants were then replanting in trays in the same perlite where they were previously grown. A week after infection each tray was inoculated with a single BCA suspension. The BCAs used were: *Rahnella aquatilis* strain 36; *Pseudomonas fluorescens* strain 57, *Serratia marcescens* strain 59 and *Bacillus amyloliquefacens* strain BO7. The final

concentration of each BCA was $2.5*10^5$ CFU/ml of perlite and each BCA was diffused on the roots by irrigating each tray with 2.5 l (the total volume retained by the amount of perlite of each tray) of a bacterial suspension. The inoculum was obtained following the protocols described by Palmieri et al. (2017)

The compared treatments were:

1. Negative control: untreated plants;
2. Positive control: plants infected with *Foc*0 and untreated with BCAs
3. Ra: plants infected with *Foc*0 and treated with *Rahnella aquatilis*
4. Pf: plants infected and treated with *Pseudomonas fluorescens*
5. Sm: plants infected and treated with *Serratia marcescens*
6. Ba: plants infected and inoculated with *Bacillus amyloliquefacens*

Each treatment was replicated three times

During the experiments, the plants were supplied with an appropriate nutrient solution every 7 days and with water when needed. The first symptoms on Cr5 and on the P5 control (plant infected by *Foc*0 but not inoculated with BCAs) appeared two months after inoculation. Subsequently, the disease progress was weekly evaluated by assessing the number of died Cr5 plants and the number of P5 plants with yellowing symptoms. Four months after inoculation the plants were explanted and for each treatment the wet and dry weight of either whole plant and roots as well as number of plants with xylematic lesions and length of lesions were assessed.

2.2.2 Pathogenic mechanisms of *Fusarium oxysporum* f.sp. *ciceris* race 0

Afterwards the same NILs were used in another experiment in order to verify the phytotoxicity of fusaric acid produced by *Fusarium oxysporum* f. sp. *ciceris* race 0. The phytotoxicity of fusaric acid was compared to the known phytotoxicity of EDTA (Hernández-Allica et al. 2007) in solutions at pH 8 by using as control distilled autoclaved water; so the tested solutions were:

1. Distilled and autoclaved water
2. EDTA 0.5 mM
3. EDTA 1 mM
4. EDTA 2 mM
5. Fusaric acid 0.15 mM
6. Fusaric acid 0.30 mM
7. Fusaric acid 0.60 mM
8. Phosphate Buffer Solution 0.1 M pH 8

In the experiment 3 chickpea plants of each genotype and thesis were used; to this aim seeds were seeded in plastic trays filled with perlite and when the emerged plants reached the 3rd real leaf stage (about 9 cm of height) they were explanted and the roots were cut during their immersion for 5 minutes in the testing solution. Following, each plant was transferred in a 50 ml Falcon tube, the roots were covered with hydrophilic cotton and 10 ml of testing solution was added to the tube.

The plants were kept at room temperature (22°C - 25°C) and after 24 h the appearance of yellowing was assessed. Then, plants were stored at -20°C in the dark to be following used in order to quantify the chlorophyll content.

To quantify the chlorophyll about 0.05 g of leaf per plant were blended and incubated in 10 ml of acetone 80% for 12 h at 4°C in darkness. After incubation, the extracted sample was filtered to remove leaf debris and used in spectrophotometric quantification. To quantify chlorophyll a and chlorophyll b the samples were read at wavelengths of 663 nm, and 645 nm, respectively.

3. Results

3.1 Landraces characterization

3.1.1 Controlled condition pathogenicity test to evaluate the resistance of chickpea landraces to *Fusarium oxysporum* f. sp *ciceris* race 5

In the growth chamber at IFAPA (Cordoba), different experiments in controlled condition were conducted to evaluate the genetic resistance of landraces to *Foc*5.

For each experiment data about the number of death plants, plants with symptoms and healthy plants were collected. These data were used to draw the disease progress curve and to calculate the Area Under the Disease Progress Curve (AUDPC). This index was used to compare the progression of the disease during two different tests and to evidence the presence of susceptible or resistant phenotypes.

According to the pathogenicity scale proposed by (Castro et al. 2010), among the 18 tested landraces, n. 203 and n. 76 are resistant to the pathogen, landraces 73, 83, 99, 111, 125, 147, 148, 241 and 245 have an intermediate degree of resistance, while other remaining ecotypes are susceptible to *Foc*5 (Table 2). Anyway, AUDPC shows that, in landraces with intermediate resistance, the disease develops with different speeds (Fig. 7). In fact, in landraces 99 and 111 the disease develops more slowly than in the others intermediate resistant landraces. Basing on AUDPC, the disease advancement is comparable to both susceptible and intermediate resistant landraces, except for one landrace. In landrace 245, the disease reached the maximum damage in less time with an AUDPC comparable to that of the susceptible landraces. The susceptible populations, except for landrace 184, had a greater effect than 245 to slow down disease development.

3.1.2 Field trials to evaluate the resistance of chickpea landraces to *Fusarium oxysporum* f. sp. *ciceris* race 5

To better evaluate the genetic resistance to *Foc*5 in landraces two pathogenicity test in field conditions were conducted: one in the experimental field of IFAPA (Cordoba) and one in the experimental field of AGROVEGETAL (Escacena del Campo). The landraces were sown in January at IFAPA and in March at AGROVEGETAL.

As regard the disease development, results of the two tests differ for some landraces according to the different localities and sowing time.

Results of field test conducted in Cordoba on landraces sown in January (winter sowing) show that landraces 111, 64, 148 and 160 developed an intermediate degree of disease severity, while all the other landraces were poorly or not affected by *Foc*5 (Fig. 8). The results of field pathogenicity tests conducted in Escacena del Campo (Huelva) on landraces sown in March (spring sowing), as it was expected, show that landraces are generally more affected with respect to those sow in January (winter sowing). In fact, landraces 73 and 148 were seriously affected by the pathogen, landraces 76 and 203 resulted resistant, while the others developed an intermediate degree of disease severity (Fig. 9). These results partially agree with those obtained in controlled condition pathogenicity tests, and the incongruity can be mainly attributed to environmental factors.

3.1.3 Field trials to evaluate the resistance of chickpea landraces to *Ascochyta rabiei*

Pathogenicity test shows that among the eighteen Italian landraces tested for resistance to A. *rabiei* only n. 62 (*Cercemaggiore*) has a degree of resistance comparable to that of the resistant control used (ILC 3279). Landrace 183 (*Cercemaggiore nero*) which is the only desi type landrace shows also a good degree of resistance. Five landraces are clearly susceptible to the pathogen in comparison to the susceptible control *Blanco Lechoso*, the remaining landraces show an intermediate degree of resistance (Fig. 10).

These data partially confirm results of genetic analysis conducted on these landraces. Analysis by molecular markers, associated with two QTLs conferring resistance to *A. rabiei* located on chromosome 4, indicates that landraces 62 and 245 should be resistant to the pathogen while 184 should be partially resistant. Contradiction between genotypic results of landraces 245 and its phenotype is due to the high quantity of QTLs controlling this trait (Gaur et al. 2012).

3.1.4 Agronomic traits

In field condition, the seed germinability was up to 80% for all the lines except for 125 that shows 60% of seed germinability.

Morphological characterization shows that among the studied landraces seven have medium erect growth habit, ten have a not properly erect growth habit and only landrace 245 (*Capracotta*) shows an erect growth habit (Table 3). The erect growth habit is an important agronomic trait for mechanization of the crop and for escaping the weeds in winter sowing (Rossini 2008). By the other side a prostrate growth habit indicates a low degree of selection in the population, in fact, the prostrate growth habit is associated to the wild relative, *Cicer reticulatum*, of chickpea and to primitive populations (Rubio et al. 2004).

Flowering time is another important trait that influences the productivity of chickpea. Early flowering allows to anticipate and to better take advantage of the production cycle, but this trait is an adaptation to equatorial or tropical latitudes, so that it is uncommon in population selected in the Mediterranean area (Siddique and Loss 2003; Rubio et al. 2004).
In the average, in all landraces flowering started 83 days after sowing, while it started 79 and 67 days in landraces 237 and 203, respectively (Table 2).

3.1.5 Genotypic characterization

Genotypic analysis was performed using the markers listed in Table 1 analysing DNA extracted from a bulk of leaflets collected by five different plants for each landrace. In general, all TA markers evidenced a high polymorphism with TA78 and TA142 markers resulting the highest and the lowest polymorphic SSR, respectively. Generally, the SSR markers analysis shows a high grade of heterozygosis in the examined ecotypes it means that there was a low degree of selection in all the landraces.
The analysis with marker associated to genes conferring resistance to *Foc*5 (Table 3) shows the presence of 230 bp and 233 bp bands of TA 27 SSR in the resistant population. The 230 bp allele appears in two populations with intermediate resistance and in two susceptible populations, while the 233 bp allele appears in three susceptible populations and in a population with intermediate resistance. For the TA 59 SSR the resistant genotype shows one allele of 225 bp. This allele appears in two susceptible population and two population with intermediate resistance. It also appears associated with TA 27 230 bp allele in one landraces with intermediate resistance to the disease, while it is associated to the TA 27 233 bp allele in one susceptible population.
The flowering markers FM5 shows the presence of a band of about 320 bp in landraces with earliest flowering date, the same band appears only in another landrace, while others shows a band of about 300 bp or 350 bp
Genetic comparison of Italian landraces with Spanish cultivars shows the presence of two genetic subpopulations (Fig. 11). Among these, the first group includes fifteen Italian landraces and 2 Spanish cultivars; however, the Spanish cultivars *Chamad* – of unknown origin – and *Puchero* – originated by mass selection of Spanish germplasm – appear distinct from the Italian landraces. Two Italian landraces appear distinct from the other populations too; one of them is the ecotype 203, resistant to *Foc*5 and with an early flowering date. Other distinct ecotypes are 184 (desi types) and 160 which in pathogenicity test appear to be very susceptible to the pathogen (Halila et al. 2008).

The second population groups consist of all the Spanish cultivars including three Italians ecotypes; among these, ecotypes 245 and 62 appear similar between them and quite similar to the Spanish cultivars *Amelia* (origin unknown), *Junco* (mass selection from ICARDA germplasm), *Badil* (mass selection from ICARDA germplasm) and *Duratón* (origin unknown). Ecotype 83, with intermediate resistance to *Foc*5, appears quite similar to the Spanish chickpea varieties *Kairo* (ILC72 X CA2156), *Athena* (ILC72 X CA2156), *Fardón* (mass selection from ILC72) *Pringao* (ILC72 X CA2156), *Saborio* (ILC72 X CA2156), *Juano* (ILC72 X CA2156), *Patio* (ILC72 X CA2156) and *Bagdad* (CA2156 X ILC72). Conversely Ecotype 83 was different from line ILC 3269 which is a direct descendent of ILC72. These results suggest that landrace 83 is related to the Spanish variety CA2156.

3.2 Plant – pathogen – BCA interactions

3.2.1 Effect of BCAs on different genotypes

To investigate the effect that BCAs have on different genotypes, resistant or susceptible to *Foc*0, two NILs were infected with the pathogen and then treated with four BCAs. The Two NILs differ in a trait of about 46 Kbp of chromosome 5, in which is located the gene conferring resistance to *Foc*0.

Data from the first experiment show the ability of PF, RA and SM to promote the growing of chickpea (Fig. 12) and to reduce the invasion of tissues by the pathogen (measured as the length of the xylematic lesions). To this aim the most effective seems to be *Pseudomonas fluorescens* (Fig. 13). A second experiment conducted using only *Pseudomonas fluorescens* and *Rahnella aquatilis* shows the effectiveness in avoid symptoms appearance in the susceptible genotype by *Pseudomonas fluorescens* (PF) (Fig. 14), while *Rahnella aquatilis* (RA) is more effective as growth promoting bacteria (Fig 15). In fact, when infected by *Foc*0 and treated with one of these bacteria, the susceptible genotype (P5) did not show yellowing symptoms. Moreover, by analysing data on length of xylematic lesions, length of the stem and number of plant with xylematic lesion an effective biocontrol activity was observed for PF but not for RA. In addition, the last one seems to be involved more in symptoms attenuation by stimulating root growth then in plant protection as PF, which avoid the pathogen contact with the plant. Data shows also the effect played by the density of inoculum of BCAs.

3.2.2 Pathogenic mechanism of *Fusarium oxysporum* f.sp. *ciceris* race 0

Since *Foc*0, together with race 1B/C, belong to the yellowing pathotype and this two races are the only ones producing fusaric acid, the role of this metabolite in the infection process was investigated. Fusaric acid is a fungal metabolite with celantic ability (Palmieri 2016). To this aim the two NILs were treated with different amount of fusaric acid and other molecules and the chlorophyll of treated plants was quantified.

Results about phytotoxicity of fusaric acid suggest an effective involvement of this molecule in the yellowing symptom produced by *Foc*0. In fact, on P5 treated with fusaric acid the yellowing is clear while it doesn't appear on P14 treated with the same molecule. This subjective evaluation is supported by data resulting from chlorophyll quantification (Fig.16 and Fig. 17) where the quantity of chlorophyll a and b in the resistant genotype (P14) treated with subtoxic (0.15 mM) or toxic (0.3 mM) concentration of fusaric acid is comparable with that of the control. P14 lost chlorophyll only at a concentration of fusaric acid that is the double with respect to the phytotoxic reported concentration. After results a bioinformatics analysis was conducted on the genome of chickpea, using the IGV 2.3.68 software to visualize the trait of about 46 Kbp of chromosome 5, in which the two lines differs. In this trait were found a different amount of sequences related whit resistance, but also a MATE efflux like protein (Accession number XM004499824). This type of proteins is responsible for metals and xenobiotics compound mobilizations and are found to be associated in the response of resistance to biotic and abiotic stresses in *Arabidopsis thaliana* (Tiwari et al. 2014).

4. Discussion and Conclusions

Chickpea is an autogamous, cleistogamous plant, it means that the androgynous flower self-pollinated before the opening of the corolla (Singh and Diwakar 1995). For this reason, despite the large morphological variability, the chickpea plant shows a small genetic variability especially in populations more selected as cultivars (Millan et al. 2006).

Cultivars are commercial population of cultivated species, breeded to reach the best yield performances and a high degree of homogeneity in an industrial scale agriculture. A landrace is a population of a cultivated living species that was selected in a defined geographical area, for characteristics conferring adaptability and best production performance, especially in areas of selections. By this definition, the process by which the landraces are selected is based only on the obtaining the biggest productivity in determined environmental condition (Negri 2003). Thus, landraces have a low degree of variability and are highly susceptible to pathogens which could appear after their selection. For this reason landraces represent a degree of biodiversity which is missing in cultivars and an additional economic resource for marginal areas (Negri 2003; Crinò and Saccardo 2008).

Resistance to pathogen could be of two kind: horizontal or vertical. Resistance to *Foc*5 is a vertical resistance conferred by a major gene located in a gene cluster which also contains the genes for resistance against races 3, 0, 4, 1 and 5 (location order on chromosome 2 between the two SSR TA27 and TA 59). Furthermore, this resistance is poorly influenced by environment. Genetic resistance to *A. rabiei*, instead, is a horizontally resistance, controlled by a multitude of genes normally located in the same chromosomal locus (Quantitative Trait Locus - QTL), this resistance is also influenced by environment in a more or less incisive degree.

The results presented in this work confirmed the high susceptibility of the landraces analyzed to the two main pathogens affecting chickpea in the Mediterranean basin, but at the same times these results evidenced the degree of biodiversity existing between landraces selected in a small area, not only in response to the resistance to diseases, but also to other agronomic traits.

The presence of traits as resistance to fusarium wilt (landraces 76 and 203), resistance to ascochyta blight (landraces 62, 184 and 245), erect growth habit (245) and early flowering (203), enhance the potential of these landraces as a good starting plant material for development of new cultivars with these desirable characteristics. The same traits could be introduced in the other landraces in a perspective to valorize and preserve them.

Data of field tests shows that environment and cultivation strategy, such as an anticipation of sowing time, can play an important role in the appearance and development of diseases. In fact,

a winter sowing time could significantly attenuate the severity of fusarium wilt, but it exposes the crops to the risk of ascochyta blight, so in this case it is very important to use landraces resistant to the latter disease if we not chose to use genetically improved material.

Genetic analysis highlights the degree of variability present in the landraces tested in the present research and simultaneously provides important tools for breeding programs using this important germplasm. Additionally, it is curious to note that the historic past of Southern Italy as an administrative region of the Crown of Aragon left a trace in the genetic similarity between some Italian landraces and other Spanish cultivar.

The use of BCAs on two NILs shows a good effectiveness in the protection conferred to the crops by these bacteria since susceptible genotypes did not develop fungal disease symptoms being comparable to the uninoculated control. Results suggest that the two biocontrol bacteria, because of their possible mechanisms of action, could efficiently prevent the infective process by colonization of plant surface/wounds or could interfere with the disease development by production of antifungal compounds and/or induction of resistance in the host tissues. However, research should be carried out to elucidate these hypotheses.

Chlorophyll quantification in plants treated by fusaric acid and EDTA reinforce the hypothesis of a direct involvement of fusaric acid in the infective process, as a chelating agent of cations causing the yellowing symptoms. The genotype susceptible to *Foc*0 results more sensitive to fusaric acid, suggesting a correlation between this sensitiveness and the susceptibility of the plant to the pathogen. Fusaric acid has a strong chelating activity (Palmieri 2016) which could subtract cations to the plants, directly or by immobilizing them in the soil; for this reason we can speculate that gene coding for proteins involved in cations mobilization could be also involved in resistance mechanisms to this pathogen.

Landraces play an important role in valorization of inner area, and reintroduction of chickpea cultivation in the agricultural system could represent an economic advantage in some marginal agriculture. Anyway, the use of landraces is limited by their susceptibility to the main pathogens, such as *Fusarium oxysporum* f.sp. *ciceris* and *Ascochyta rabiei*, of this crops but the present research evidenced as a good source of genes could be useful resource to be used in the breeding of the same landraces and/or of some important cultivars. This could create an economic advantage also for little farms since the availability of selected and well characterized plant material, which simultaneously respects the characteristic of a traditional product, could be stimulate cultivation and produce economical advantage.

Finally, since the exclusive use of genetic resistance and agronomic practice are sometimes not appropriate to protect the crop by plant diseases, the use of BCAs, especially against soilborne

pathogens, as evidenced in the present study, could be a further tool to stimulate cultivation and management of chickpea. However, an optimal use of BCAs needs further studies to better understand the mechanisms of action involved in the action of these useful organisms.

In conclusion the present study supplies new and important scientific information on agronomic and genetic characteristic of new Italian chickpea landraces with particular attention to their resistance to some dangerous fungal pathogens as well as on the possible use of genetic tools in combination with biocontrol agents for an ecocompatible control of fungal diseases. The genetic resistance evidenced in some landraces open the way to further research aimed at a better characterization of such genes to be used in improving resistance of chickpea to fungal pathogens.

5. Tables

Table 1 – Main markers used for the genetic characterization of the chickpea landraces

Marker	Range	Number of founded Alleles	Number of Unique Alleles	Most Frequent Alleles (> 0.2)	Commons Alleles (0.03-0.2)	Rare Alleles (<0.03)	Number of Landrace with only one Allele	PIC
TA11	220-262	14	2	0	12	2	3	0.826081894
TA14	242-278	11	3	0	8	3	3	0.825823602
TA27	218-248	**11**	4	0	7	4	1	0.838679923
TA59	222-273	12	5	1	6	5	6	0.799276302
TA78	191-236	15	3	0	12	3	0	0.884902889
TA113	169-217	11	5	1	10	0	9	0.808151216
TA135	187-199	5	1	3	2	0	5	0.595293399
TA144	230-254	8	1	2	5	1	0	0.733092594
CaGM07992	300-350	3	1	2	1	0	17	0.340620468
H2I20	180-230	5	3	2	3	0	16	0.38865
FM5	300-350	3	0	1	2	0	16	0.353475
TA143	150-160	2	0	1	1	0	16	0.213285617

Table 2 - Association between date of flowering, resistance to *Foc*5 and molecular marker alleles founded in the examined chickpea landraces

Accession	Day to Flowering	Resistance to *Foc*5*	TA27	TA59	CaGM 07992	H2I20	FM5	TA142
62	ND	S	236-239	246-255-273	300	200	300	150
64	87	ND	221-233-242	225-234	300	200	300	150-160
73	86	I	221-227-239	231-234-237	350	220	300	150
76	87	R	227-230	228-234	300	200	300	150
83	88	I	221-236-239	243-246	350	220	350	160
97	86	S	221-239-242	231-234	350	200	300	150
99	84	I	221-227	231-234-237	300	200	300/350	150-160
111	84	I	221-227-230	225	300	230	320	150
125	84	I	221-233-236	234	350	200	300	150
147	84	I	239-242	222-225-234	350	200	300	150
148	84	I	221-227-233-236	228	300	200	300	150
160	ND	S	221-224-230	237-240	300	220	300	150
184	87	S	221-236-239-242	246	300	200	300/350	150
203	67	R	230-233	225	350	220	320	150
228	84	S	221-239-242-245-248	234	320	180	300	150
237	79	S	221-230-233-236	234-237	300-350	190-200	300	150-160
241	83	I	218-221-227-239	225-234-237	300	200-220	300	150
245	87	I	236	252-255	300	200	300	150

*Basing on results of controlled condition pathogenicity test R = Resistant (less than 10% of death plants), I= Intermediate resistant (between 11% and 89% of death plants) and S=Susceptible (more than 90% of death plants)

Table 3 - Italian chickpea landraces used in this study; the growth habit was evaluated using a 0-3 empirical scale (from 0, corresponding to a landed growth habit, to 3, corresponding to an erect one).

Accession	Origin (Town)	Province*	Coordinates	Growth habit	Germination	Seed size (g of 100 seeds)	Type
62	Cercemaggiore	CB	41°28′N 14°43′E	2	90%	27.1	Kabuli
64	Cercemaggiore	CB	41°28′N 14°43′E	1	80%	36.7	Kabuli
73	Salcito	CB	41°45′N 14°31′E	1	90%	37.3	Kabuli
76	S. Elia a Pianisi	CB	41°37′N 14°53′E	2	100%	38.4	Kabuli
83	Casacalenda	CB	41°44′N 14°51′E	1	90%	36.2	Kabuli
97	S. Angelo del Pesco	IS	41°53′N 14°15′E	2	100%	35.3	Kabuli
99	Venafro	IS	41°29′4″N 14°2′45″E	2	90%	31.7	Kabuli
111	Ripabottoni	CB	41°41′N 14°49′E	2	100%	52.4	Kabuli
125	Morrone del Sannio	CB	41°43′N 14°47′E	2	60%	48.6	Kabuli
147	Riccia	CB	41°29′N 14°50′E	2	80%	42.5	Kabuli
148	Filignano	IS	41°32′42.94″N 14°3′23.9″E	2	100%	30.5	Kabuli
160	Miranda	IS	41°39′N 14°15′E	1	90%	35.8	Kabuli
184	Cercemaggiore	CB	41°28′N 14°43′E	1	100%	21.9	Desi
203	Longano	IS	41°31′N 14°15′E	1	90%	47.9	Kabuli
228	Riccia	CB	41°29′N 14°50′E	1	100%	35.4	Kabuli
237	Montagano	CB	41°39′N 14°40′E	2	100%	34.6	Kabuli
241	Riccia	CB	41°29′N 14°50′E	2	100%	31.2	Kabuli
245	Capracotta	IS	41°50′N 14°16′E	3	90%	27.2	Kabuli

*IS= Isernia; CB= Campobasso

6. Figures

Fig. 1 - Seeds and flowers of desi type (A and B) and kabuli type (C and D) chickpea.

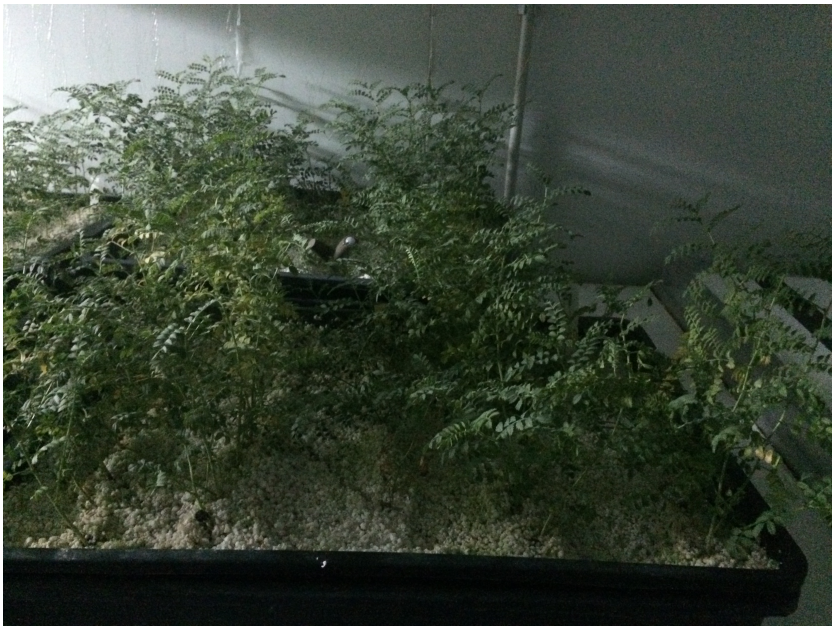

Fig. 2 - Yellowing symptoms caused by *Fusarium oxysporum* f. sp. *ciceris* race 0 (plants on the right-side)

Fig. 3 - Xylematic lesions caused by *Foc*0

Fig. 4 - Wilting symptoms caused by *Foc*5

Fig. 5 - Lesions caused by *Ascochyta rabiei* on flowers and leaves of chickpea

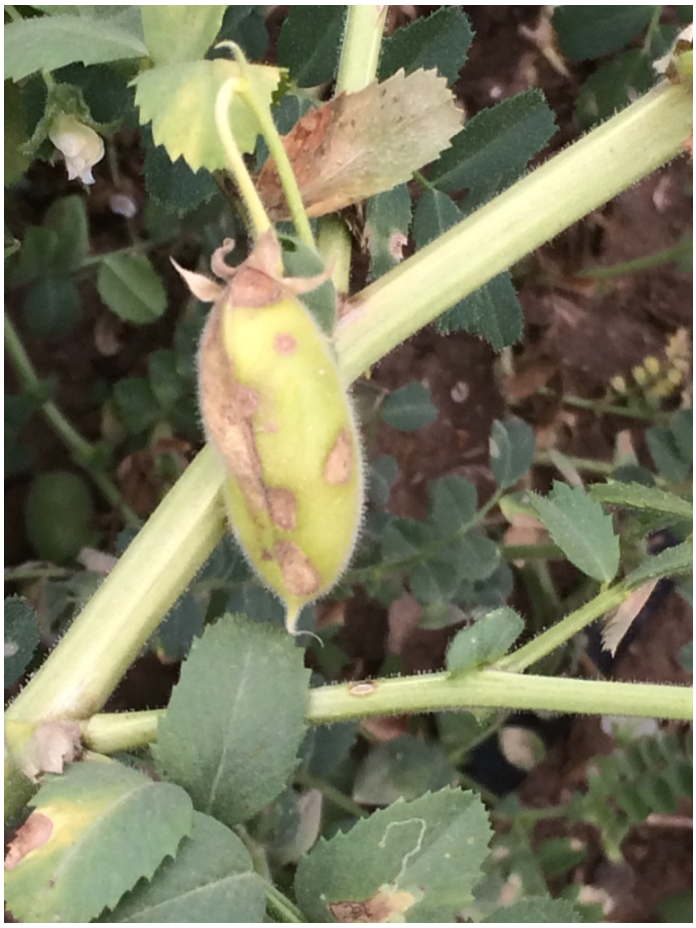

Fig. 6 - Lesions caused by *Ascochyta rabiei* on chickpea pod

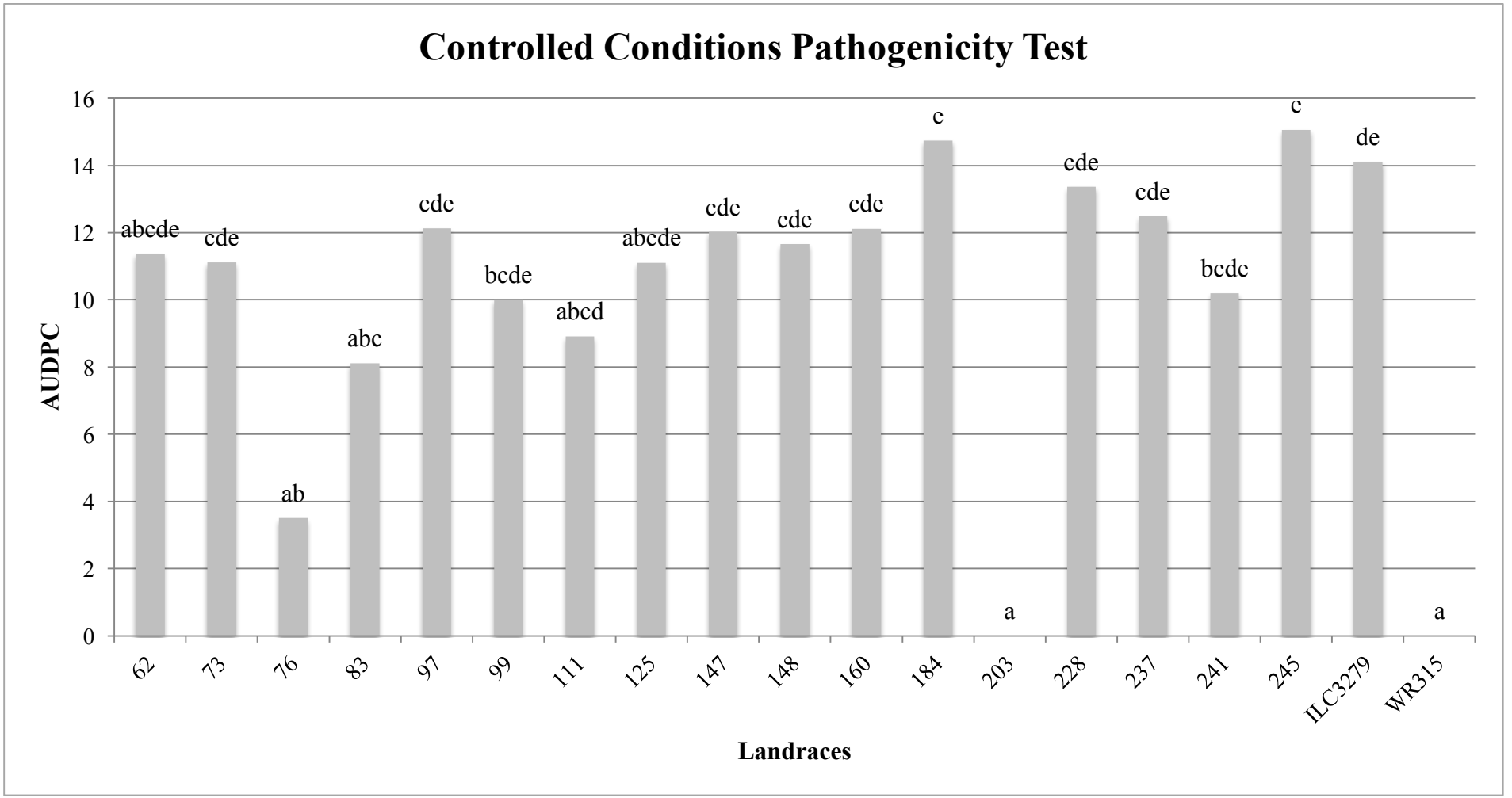

Fig. 7 - Average Area Under the Disease Progress Curve (AUDPC) obtained from the pathogenicity tests conducted in controlled conditions on chickpea landraces artificially inoculated with *F. oxysporum* f. sp. *ciceris* race 5 (*Foc*5). ILC3279 and WR315 are *Foc*5 susceptible and resistant cultivars, respectively. The ecotype M64 is not reported because of its low seed germination in perlite

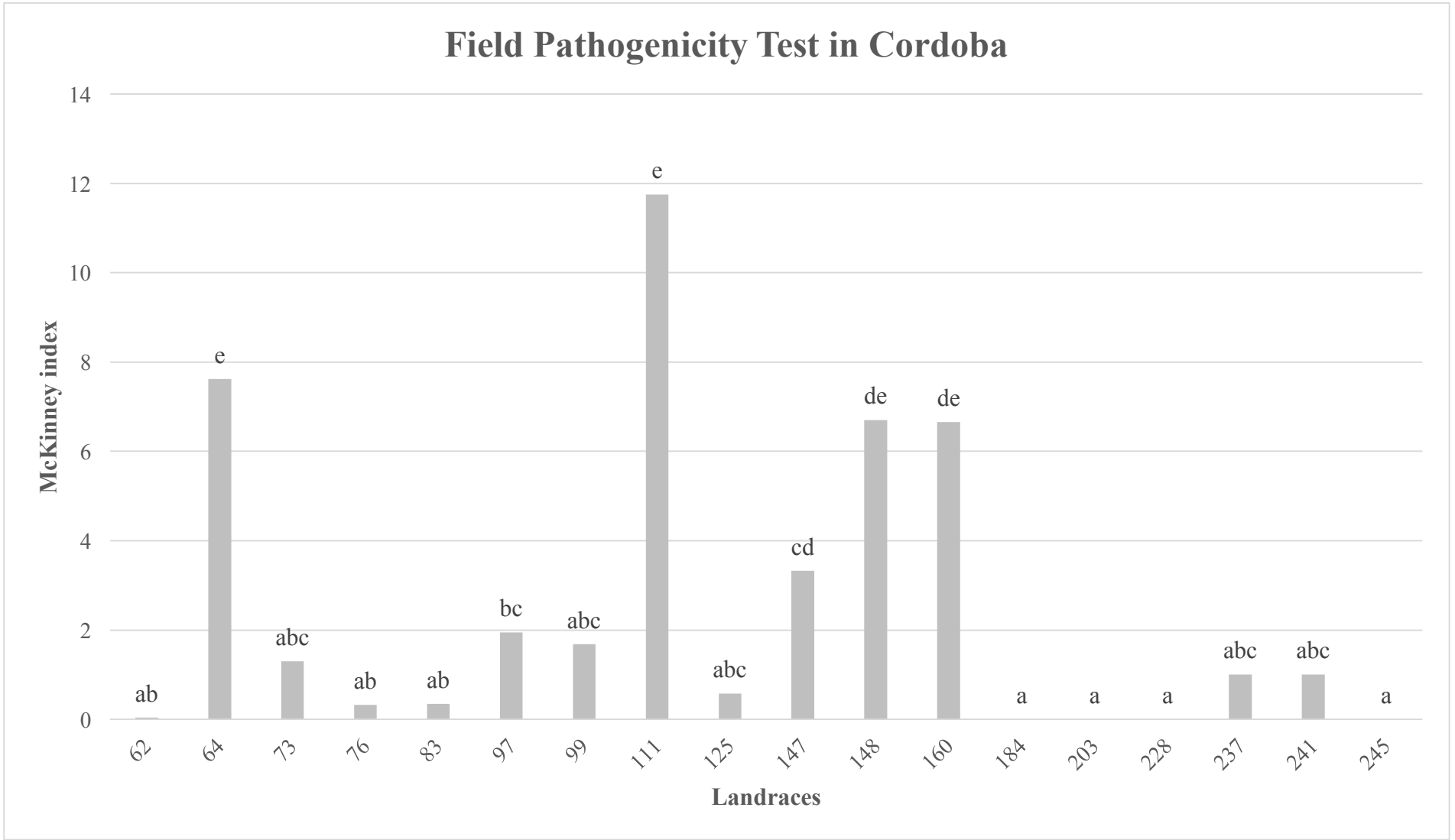

Fig. 8 - Severity of disease on the tested landraces, in the experimental field of IFAPA-Cordoba, naturally contaminated with *Foc*5. Landraces were sow in January (winter sowing).

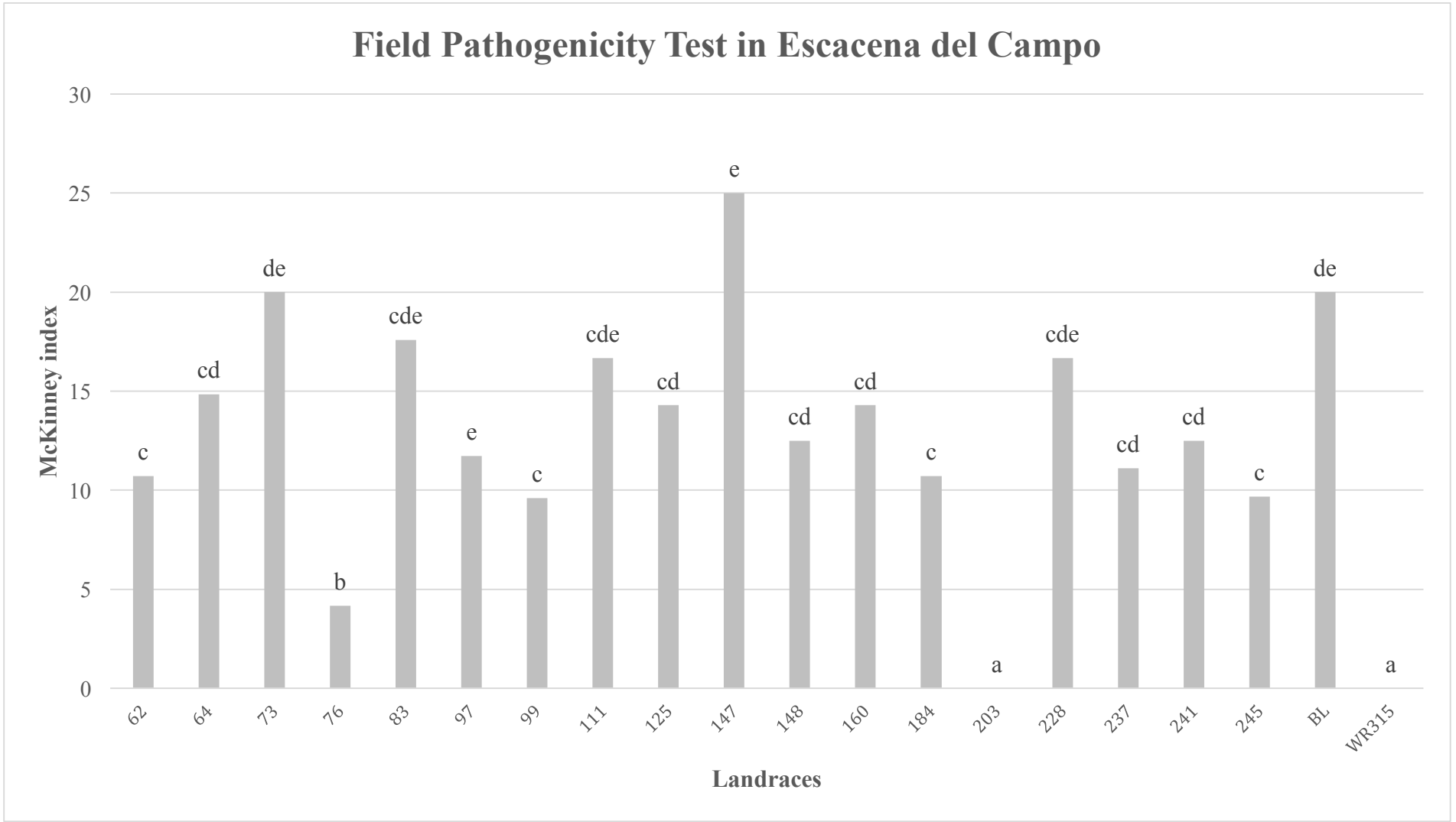

Fig. 9 - Severity of disease on the tested landraces, inn the experimental field of AGROVEGETAL- Escacena del Campo (Huelva), naturally contaminated with *Foc*5. Landraces were sow in March (spring sowing).

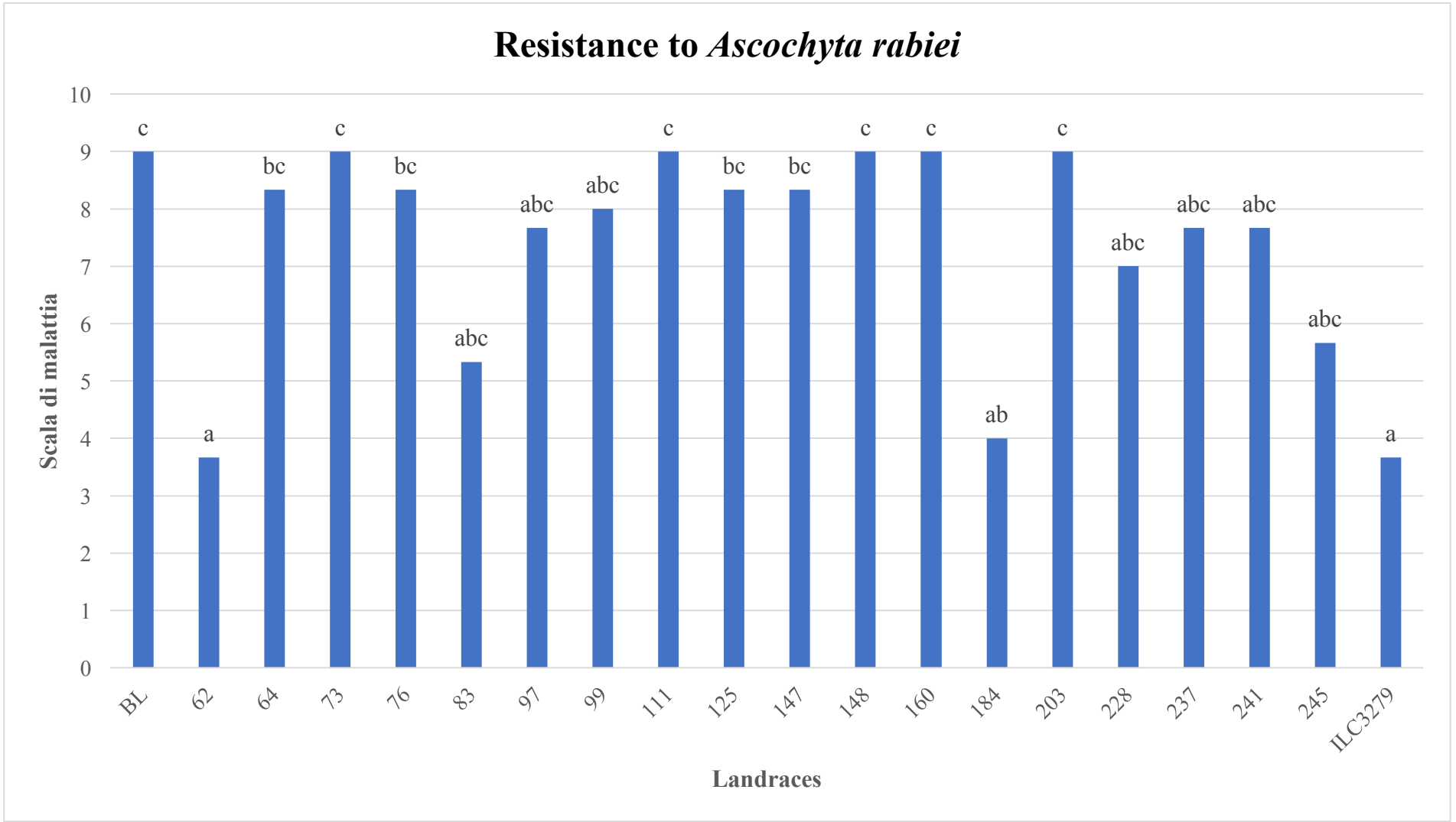

Fig. 10 - Severity of *Ascochyta blight* disease on the eighteen Italian landraces.

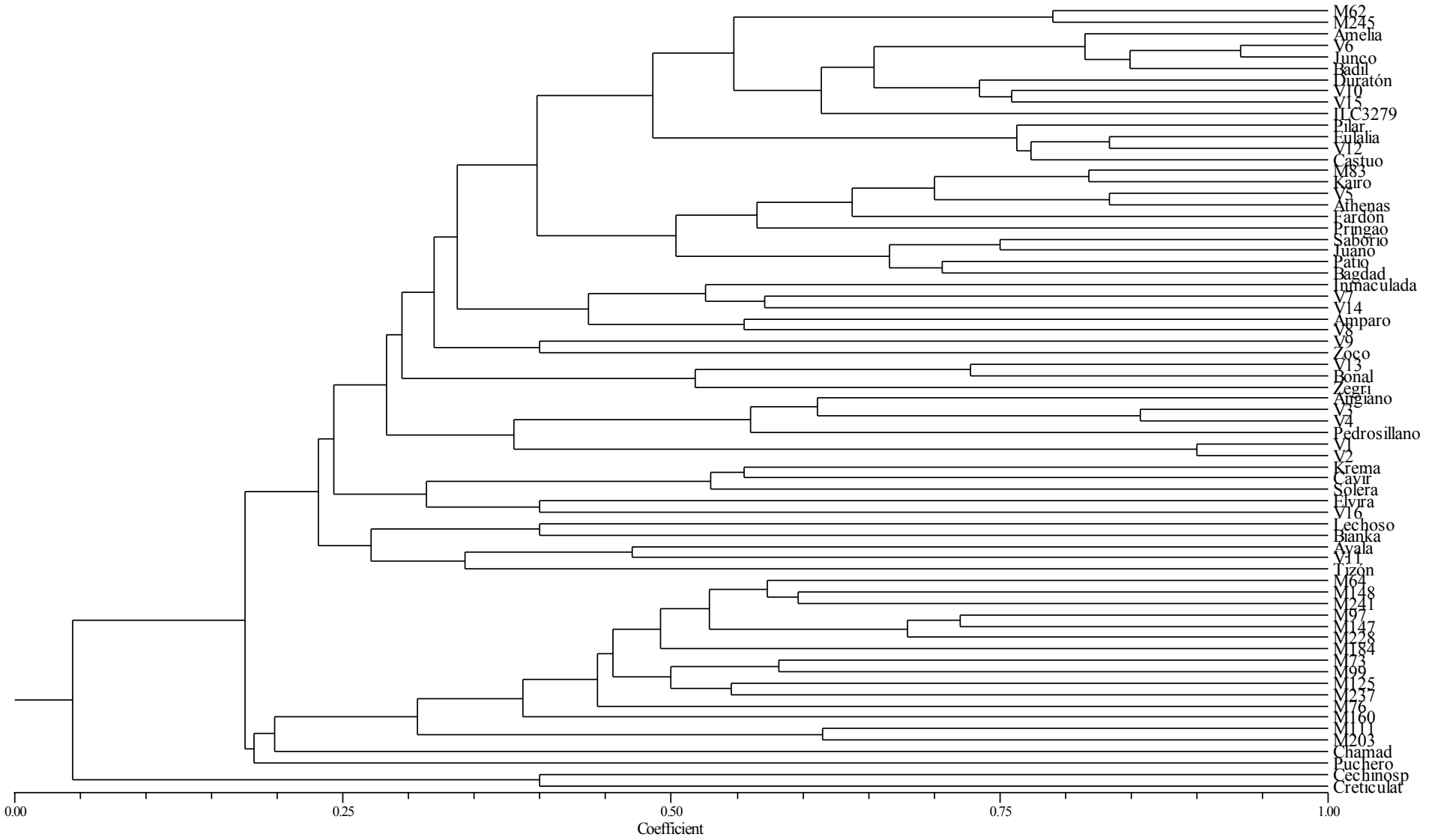

Fig. 11 - UPGMA dendrogram of Italian chickpea landraces and Spanish cultivars using 15 STMS loci and Dice Coefficient(Nei and Li 1979)

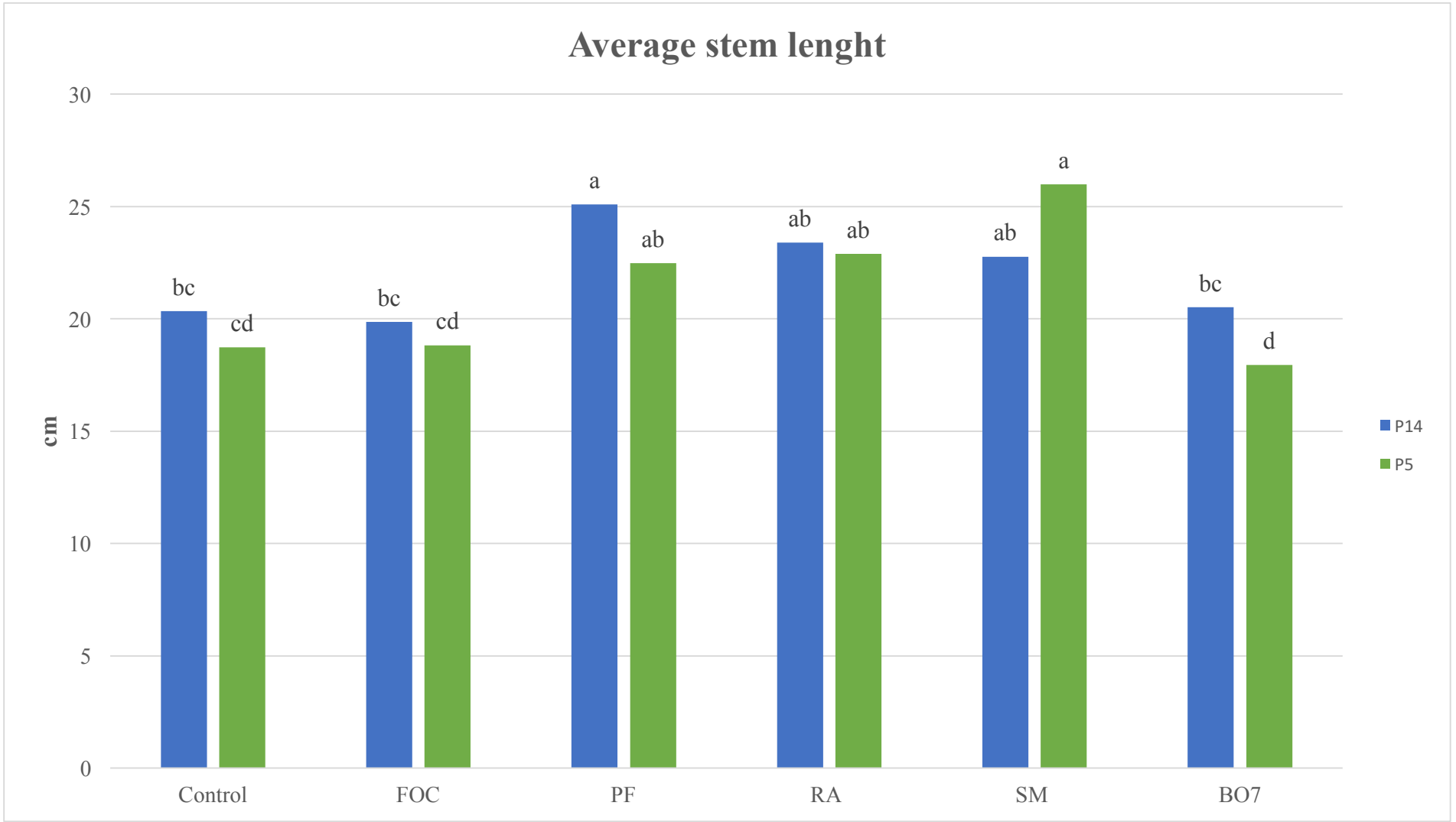

Fig. 12 - Differences in stem lenght in plant resistant to *Foc0* (P14) and susceptible (P5). The plants were treated with strains of *Pseudomonas fluorescens* (PF), *Rahnella aquatilis* (RA), *Serratia marcescens* (SM) and *Bacillus amyloliquefacens* (BO7)

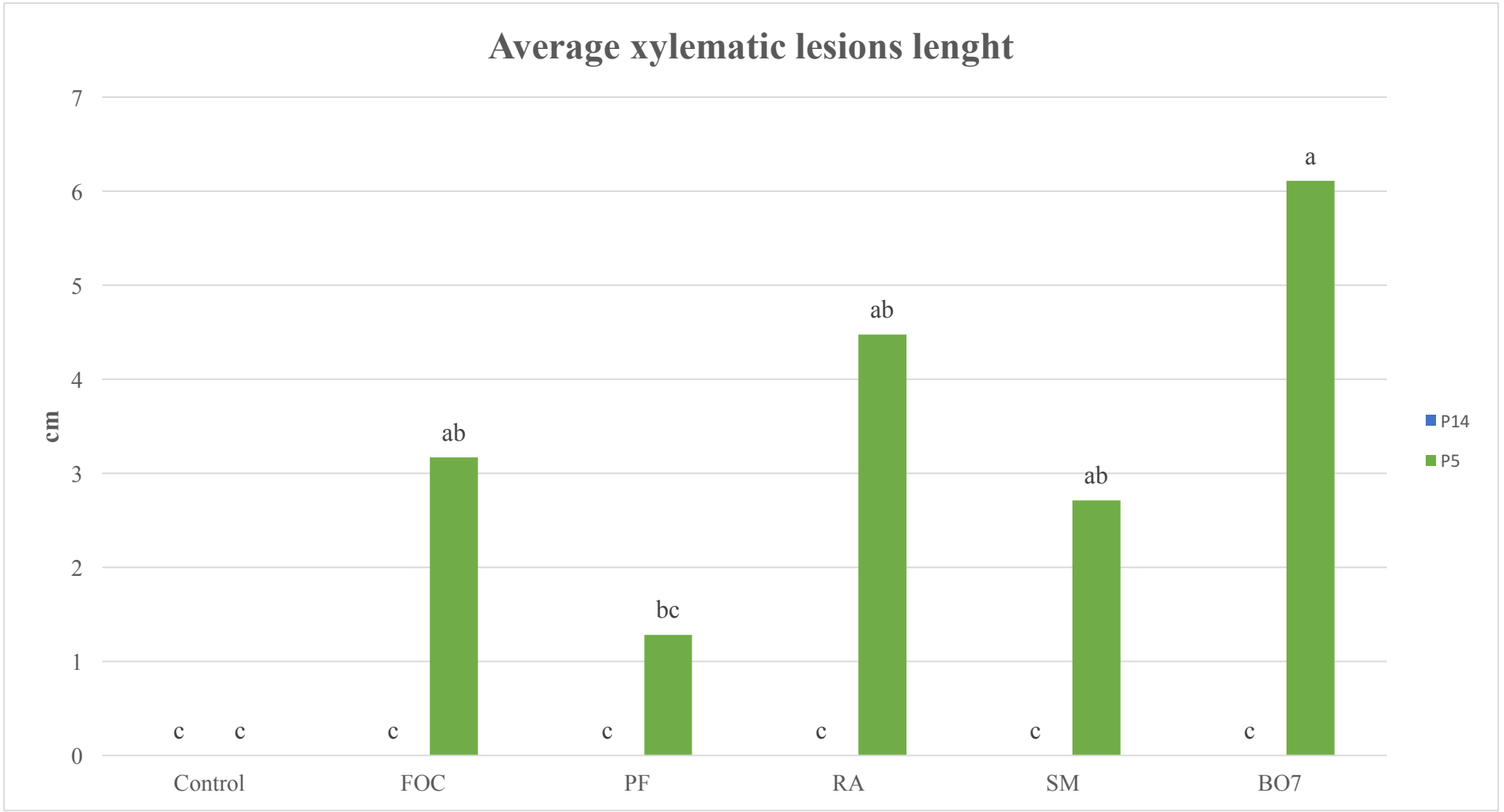

Fig. 13 - Differences in xylematic lesions lenght in plant resistant to *Foc*0 (P14) and susceptible (P5). The plants were treated with strains of *Pseudomonas fluorescens* (PF), *Rahnella aquatilis* (RA), *Serratia marcescens* (SM) and *Bacillus amyloliqiuefacens* (BO7) as expected the resistant genotype didn't show xylematic lesions so the data collected was 0 in all the plants of P14.

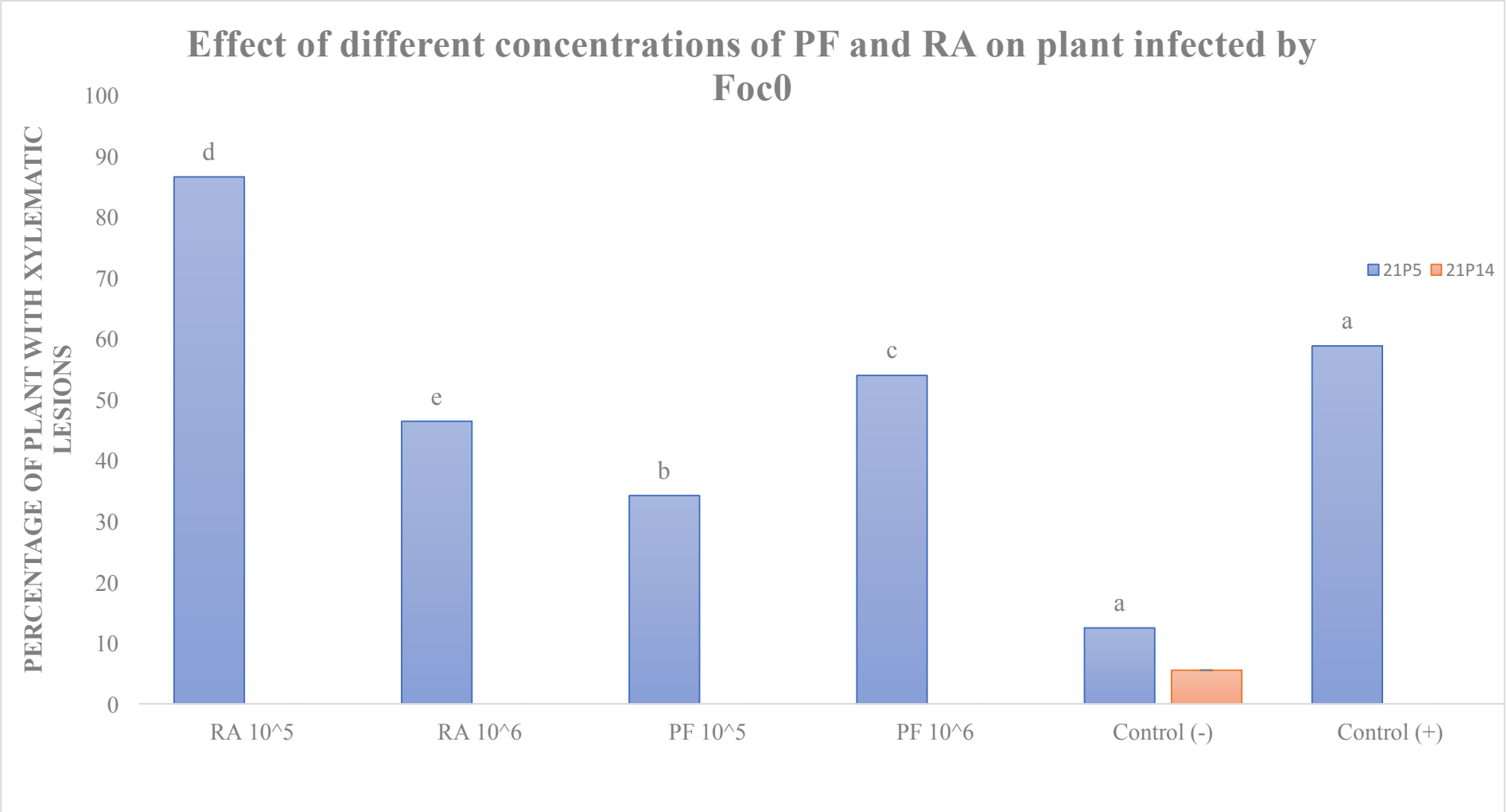

Fig. 14 - Differences in percentage of xylematic lesions in plant resistant to *Foc*0 (P14) and susceptible (P5). The plants were treated with strains of *Pseudomonas fluorescens* (PF) and *Rahnella aquatilis* (RA), at two different concentrations: $2.5*10^5$ and $2.5*10^6$ CFU /gr of perlite, as expected the resistant genotype didn't show xylematic lesions so the data collected was 0 in all the plants of P14.

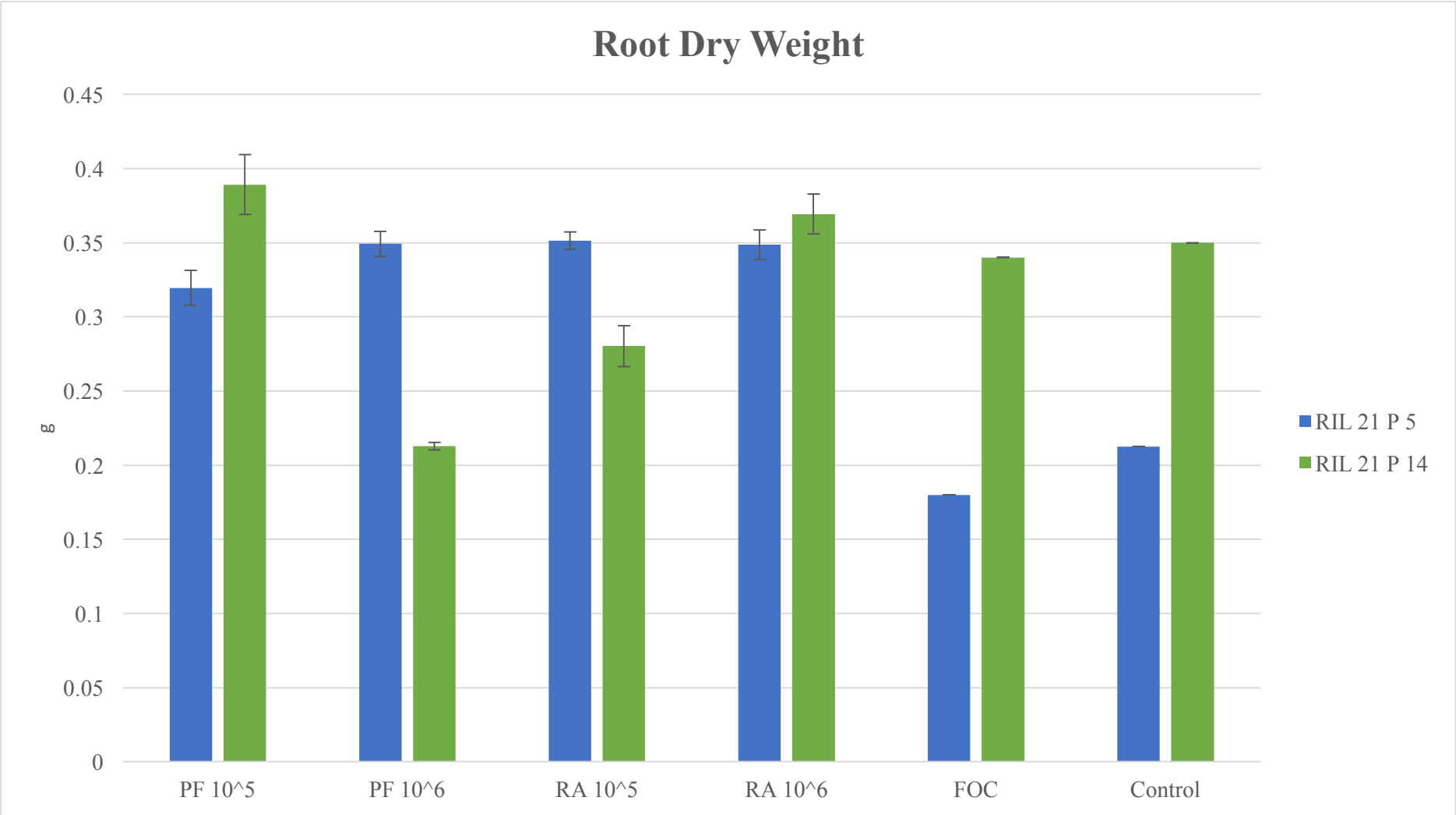

Fig. 15 - Differences in root dry weight in plant resistant to *Foc*0 (P14) and susceptible (P5). The plants were treated with strains of *Pseudomonas fluorescens* (PF) and *Rahnella aquatilis* (RA), at two different concentrations: $2.5*10^5$ and $2.5*10^6$ cfu /gr of perlite, as expected the resistant genotype didn't present xylematic lesion so the data collected was 0 in all the plants of P14.

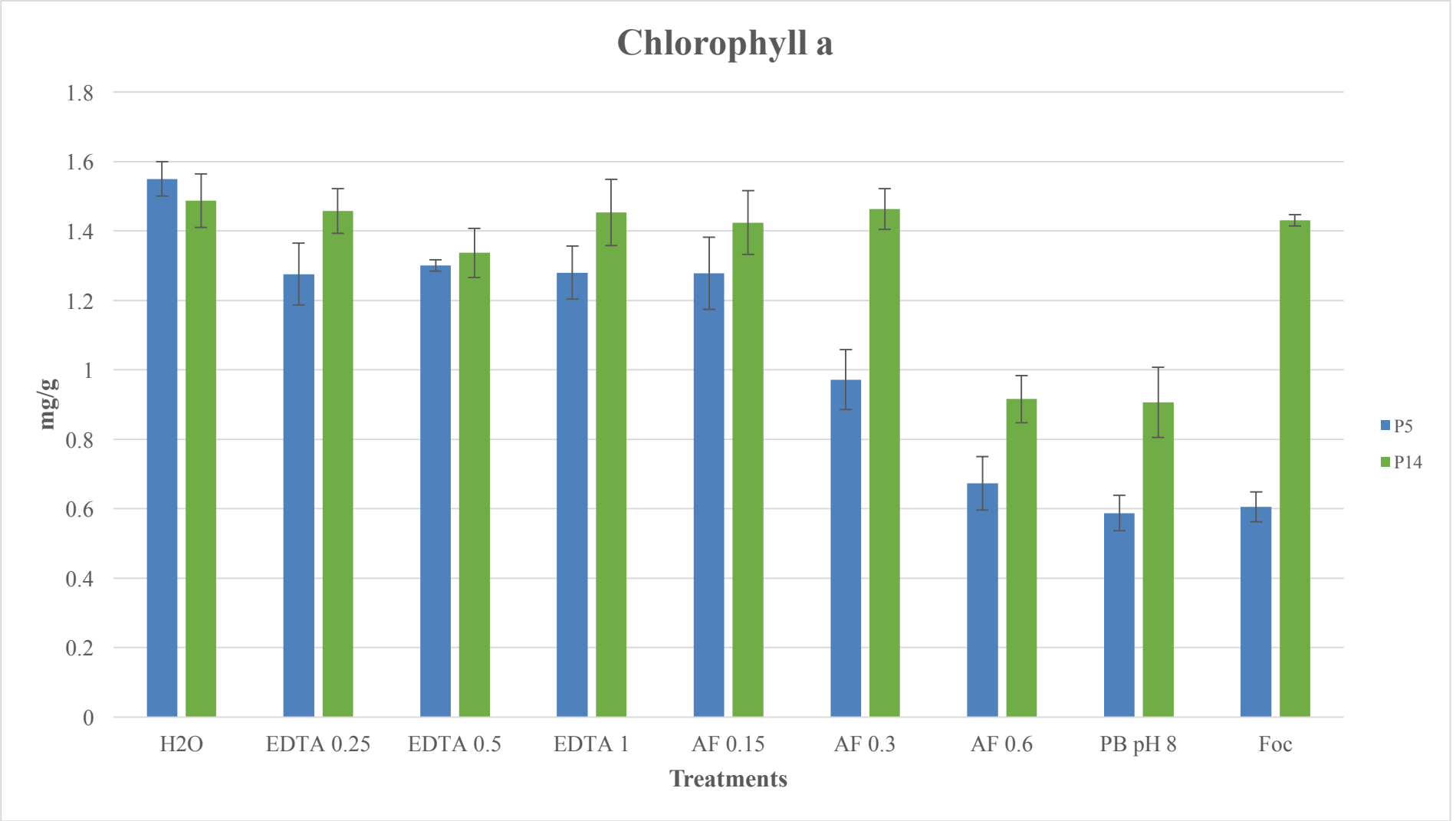

Fig. 16 - Content of chlorophyll a in plants treated with different concentration of EDTA (0.25 mM, 0.5 mM and 1mM), different concentration of fusaric acid (0.15 mM, 0.3 mM and 0.6 mm), phosphate buffer 0.1 M pH8, and infected by *Foc0.*

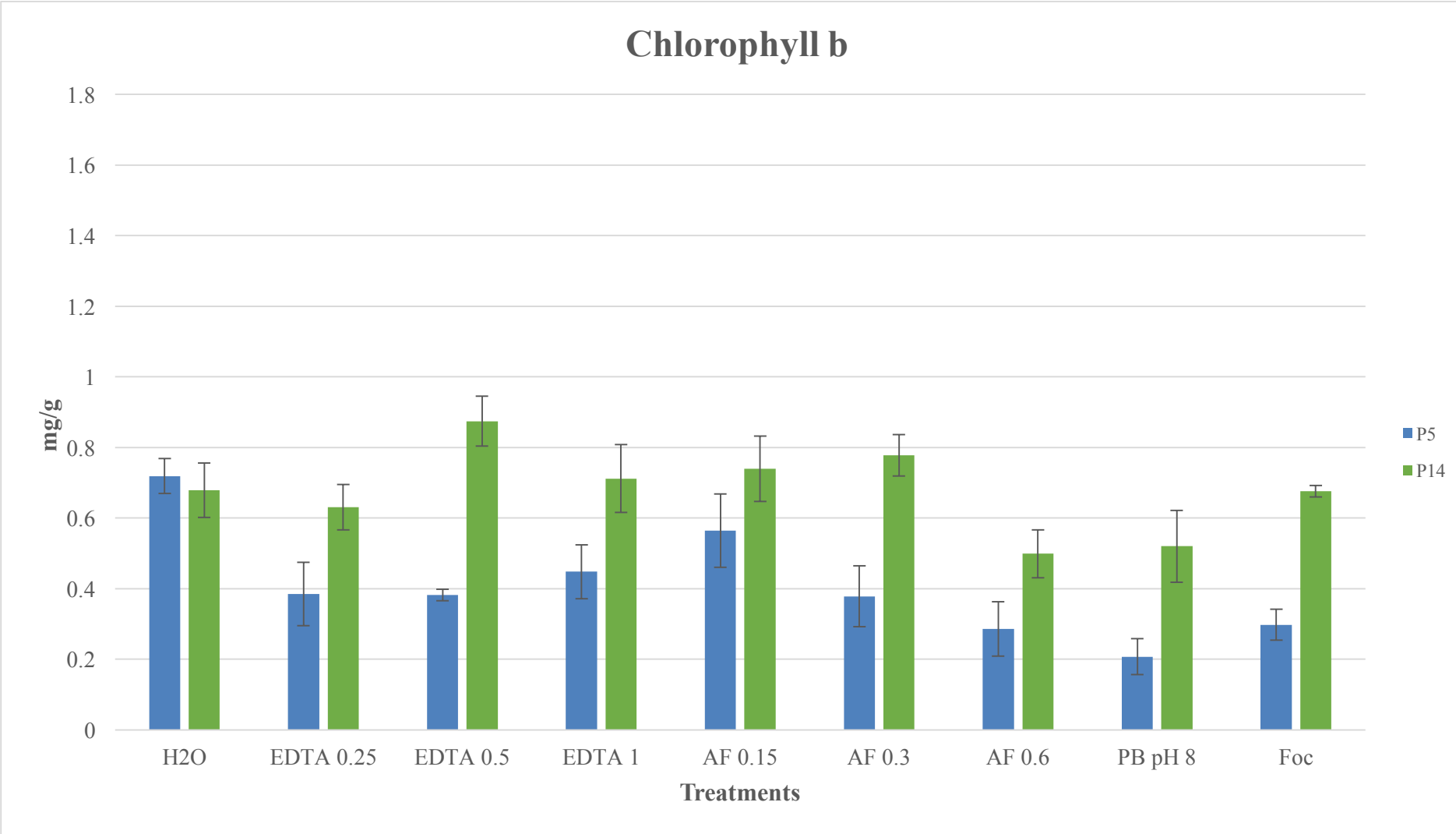

Fig. 17 - Content of chlorophyll b in plants treated with different concentration of EDTA (0.25 mM, 0.5 mM and 1mM), different concentration of fusaric acid (0.15 mM, 0.3 mM and 0.6 mm), phosphate buffer 0.1 M pH8, and infected by *Foc0.*

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