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Title: "Identification of resistant and susceptible common bean landraces in response to rust and fusarium wilt infections"

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- **Background:**

Common bean (*Phaseolus vulgaris* L.) is the most important food legume worldwide. Portugal holds a very diverse common bean germplasm consisting of landraces that resulted from more than five centuries of environmental adaptation and mass selection by farmers. Nevertheless, beans are underused in Europe, being Portugal highly dependent on imports to satisfy national intake. Diseases caused by fusarium wilt and rust fungi have great impact on yield loss and are among the main causes of this underuse. The agronomical, morphological and molecular diversity already observed in this Portuguese common bean landrace collection indicates the presence of sources of resistance not yet explored in plant-pathogen interaction research and foresees the potential identification of novel resistance and effector genes.

- **Objectives/ Purpose of the visit:**

1. Characterization of responses to an airborne and a soilborne fungus infection (rust and fusarium wilt) of a common bean germplasm collection under controlled conditions. Fungal isolates, equipment and expertise for disease screenings are available at IAS-CSIC, Cordoba, Spain.

2. Identification of sources of resistance, possessing different mechanisms of resistance to fungal infection, for inclusion on future plant breeding crossing schemes, and for future association genetic studies using the already available molecular genotyping data of the collection.

3. Collection of inoculated plant samples from contrasting landraces, regarding resistance response, for future transcriptomic analysis (eventually by RNAseq) of plant-pathogen interaction and potential identification of resistance and effector genes.

- **Description of the work**

A collection of Portuguese common bean landraces was evaluated for rust and fusarium wilt resistance under growth chamber conditions at IAS-CSIC within the group of Diego Rubiales. All seeds were surface-sterilized for 20 min in a 20% solution of sodium hypochlorite and then rinsed three times with sterile water. For the rust experiment, the protocol followed was the one described in Leitão *et al*. (2013). Seventy-six (76) common bean landraces were sown in 6x6x8 cm plastic pots filled with peat/sand [3:1 (v/v)] with one seedling per pot. Pots were placed in a growth chamber kept at 27 ± 2°C under a photoperiod of 14 h light (250 µmol/m^2) and 10 h dark, with a relative humidity of 70% and well watered during all experiments. The experiment consisted of three consecutive repetitions, each with three to five pots per landrace. For each repetition, all the plants were simultaneously inoculated by dusting 2 mg of *Uromyces appendiculatus* spores per plant (two-week-old), diluted in pure talcum powder (1:10). The final inoculum density was about 30 spores/mm^2. The inoculum was previously collected in Lanestosa, Vizcaya, Spain, and was stored at -80ºC. Plants were incubated for 24 h at 20°C in complete darkness and 100% relative humidity, then transferred to the initial growth chamber and kept at 27 ± 2°C under a photoperiod of 14 h light and 10 h dark. The infection type (IT) was scored 12 days after inoculation (DAI) using the IT scale of Stakman *et al.* (1962), where 0 represents immune: no symptoms, 1 very resistant: hypersensitive necrotic flecks with minute and isolated pustules barely sporulating, 2 moderately resistant: necrotic halo surrounding pustules small to medium in size, 3 moderately susceptible: chlorotic halo surrounding pustules medium in size, 4 susceptible: well-formed large and numerous pustules, often confluent, with no associated chlorosis or necrosis. Scores 0–2 are considered indicative of resistance and 3–4 of susceptibility. Disease severity was estimated as the percentage of leaf area covered by rust pustules and/or necrotic spots 12 DAI, on full expanded cotyledon leaves.

Similarly, components of resistance to *Fusarium oxysporum* f. sp. *phaseoli* were studied in seedlings under controlled conditions as described by Rispail & Rubiales (2014). Ninety-five (95) common bean landrace were sown in 0.5 L pots filled with sterile vermiculite (1–3 mm diameter), one seed per pot, three to five plants per landrace. Pots were placed in a growth chamber kept at 26 +- 2°C under a photoperiod of 14 h light (250 µmol/m2) and 10 h dark, well

watered during all experiment. The fungal strain was stored as microconidial suspensions at -80ºC in 30% glycerol. For microconidia production, cultures were grown in potato dextrose broth at 28ºC in a shake culture set at 170 rpm. Seven-day-old seedlings were inoculated following a modified version of the dip technique described by Haglund (1989). For this procedure, vermiculite was removed from the roots which were trimmed by a third and immersed for 5 min in a suspension containing $5x10^6$ microconidia/mL of water. Control plants were treated in the same way and were immersed in sterile water. Seedlings were planted in individual pots containing sterile vermiculite and maintained in the same growth chamber.

Symptoms were assessed every three days, from 7th to 30th days post-inoculation, with ratings value based on a disease index scale ranging from 1 (healthy leaf) to 5 (dead leaf) (Bani *et al*. 2012, Rispail & Rubiales, 2014). These data will be used to calculate the AUDPC (the area under the disease progress curve).

The most contrasting (resistant/susceptible) landraces were identified to perform a more detailed evaluation of the biotic resistance through an expression analysis of candidate resistance and effector genes. The three more susceptible and three more resistant landraces were chosen. Plant samples (roots, stems and leaves) of three plants per landrace and per condition (inoculated/24h, inoculated/96h), were collected at 24h and 96h after fusarium infection, plus the respective non-inoculated controls (control/24h, control/96h), immediately frozen in liquid nitrogen and stored at -80ºC for future RNA isolation and sequencing (RNAseq).

Finally, to determine the extent of *Fusarium oxysporum* f. sp. *phaseoli* colonization, the fungus was reisolated from the root and the basal, middle and apical stem regions of three inoculated common bean plants from three susceptible and three resistant landraces 30 days after infection.

- **Main results:**

The results are still being analyzed but it can already be anticipated that there is great variability in disease resistance among the Portuguese common bean landraces, revealed in different responses to rust and fusarium fungi infection. From the rust screening, it was verified that 55 from the 76 common bean landraces screened showed a mixed disease reaction, with more than one infection type and with variation in disease severity among plants from the same landrace. This indicates that there is some level of heterozygosity that can be useful to select the more resistant plants within these landraces. The most frequent infection type observed was 4, indicative of a compatible interaction plant-pathogen, with no macroscopically visible

hypersensitivity and with disease severity values that vary greatly, from <1 to 40 %. Sixteen landraces had plants showing low infection types (0, 1 or 2), indicative of incompatible interaction. Forty landraces had plants with leaves with chlorotic halos surrounding the rust pustules.

In the fusarium screening it was observed that, a week after the inoculation, some landraces presented disease symptoms in the primary (cotyledonary) leaves, with curled yellow margins that progressed fast to a stage where whole leaves became wilted and dry. Two weeks after the infection, plants from 5 landraces were already dead. On the other hand, 10 landraces showed plants with resistance to fusarium infection that presented no symptoms or only low levels of yellowing or discoloration on the leaves margins, revealing once more the variability present in this valuable germplasm.

When segments from the root and the basal, middle and apical stem regions from inoculated susceptible and resistant landraces were cut to reisolate the fusarium fungus, it was observed that, in the susceptible landraces, the fungus colonized the whole plant and fungal colonies were found in all regions, from root to apical stem. On the other hand, in the resistant landraces, the fungal colonies were present mainly in the roots and basal stem, suggesting that the resistance mechanisms from the plant prevented the fungus to spread from the lowest to the highest parts of the plant.

There is still a need to perform more repetitions of this screening with the same landraces to corroborate these results.

The comparative transcriptomic analysis that will be performed in contrasting landraces will help to clarify molecular pathogenicity mechanisms and will possibly identify candidate resistance and effector genes. Once these mechanisms are characterized, this germplasm can be better exploited on the development of durable resistance that will contribute to the improvement of the Portuguese common bean resistance breeding.

- **Future collaboration with the host institution (if applicable):**

As part of my PhD thesis project (co-supervised by Prof. Diego Rubiales) more visits are planned to his laboratory at IAS-CSIC, to continue the disease resistance screening of the Portuguese common bean collection.

- **Projected publications/articles related to or resulting from the STSM:**

It is expected that the completed disease resistance screening of the Portuguese common bean collection will be published in the future under the scope of my PhD thesis.

- **References**

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