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BREEDING FOR RESILIENT, EFFICIENT AND SUSTAINABLE ORGANIC VEGETABLE PRODUCTION SCDL BACAU ROLE IN BRESOV H2020 PROJECT - DEVELOPMENT OF POPULATIONS, ADVANCED BREEDING LINES AND IMPROVED GENETIC MATERIAL FOR EUROPEAN ORGANIC AGRICULTURE

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INTRODUCTION

Over the last decade, the focus on organic farming has a continue increase year by year in Europe. The total organic agricultural area of the EU was 11.9 million hectares in 2016 and is expected to grow in the coming years. During 2012-2016, the share of the ecological surface of the total agricultural area within the EU increased from 5.6% to 6.7%. The plant sector represents a small part of the ecological surface: 145,639 hectares in 2015.

The work presents the objectives and tasks of BRESOV project funded by EU in frame of Horizon 2020 research and innovation program under grant agreement Nr. 774244. SCDL Bacau acts as partner and develops researches related pre breeding and breeding of *Phaseolus vulgaris* L and also development of specific technics and methods for organic cultivation of *Phaseolus vulgaris* L, *Brassica oleracea* L, and *Solanum lycopersicum* L. in the context of rotation schemes.

Organic farming requires the investigation and enhancement of the genetic diversity to exploit the interactions between genotypes and their environments. The interest is focussed not only on yield components and traits related to quality, but also on development of root system and plants development under the impact of abiotic and biotic factors. In the context of changing climate conditions it is important to increase the efficiency of water use and actions, as well as to be able to withstand and / or tolerate the attack of pests and diseases.

The project development will be facilitated by the active involvement of farmers, consultancy services, research institutes and the food sector in various geographical / climatic contexts in Europe, Asia and Africa. As the consumption of organic vegetables has increased throughout Europe. BRESOV will offer alternative solutions for organic producers. Currently there are not available vegetables varieties developed specific to organic farming systems. The overall aim of the project is to respond to the needs of organic producers by encouraging research to develop varieties suitable for organic production, taking into account the specific needs and objectives of organic farming, such as improved genetic diversity, resistance / tolerance to pathogen attack and adaptation to various soils and climatic conditions.

MATERIAL AND METHODS

At the level of the entire consortium, joint work collections for the three investigated species were established through the contribution of each partner. Some of them were multiplied in the first year to ensure the need for seeds for all the partners.

The genetic diversity exploited within the project for the breeding of the *Phaseolus vulgaris* L. species is very wide and includes three working collections in which they were introduced: varieties, improved lines, local populations, wild relatives, to be investigated under different agro-climatic conditions.

P11_VRDS Bacau developed during 1st month and 18th month of BRESOV a study to screen **material** for complex traits related to resilience under organic agriculture. The study was designed inside de BEAN crop group and it was coordinated by UNIVPM, crop (bean) group leader.

Based on MTA, signed between UNIVPM (P7) and VRDS Romania (P11) bean genetic resources, respectively 300 Ils, 5 advanced breeding ILs and 2 parental lines were received by P11, in order to be phenotypically investigated in organic open field condition. Comparison using local varieties as control were realized. Same traits (table 1) as in case of BRESOV ILs and SBP were analyzed. These varieties were provided by P11, also investigated for WP5 objectives.

The protocol for investigation was established inside the BEAN group, agreed and implemented by P11 together to the other two partners. The structure of biological material at project level is summarized in table 2. Table 2 prenents the the working collections for first phase of the project.

Table.1. Scheme of bean collection to be invetsigated

HIPER-CORE COLLECTION (HCC) – includes ~ 80 lines:
80 lines: American wild and domesticated accessions (pure lines) from NEXT_BEAN and BEAN_ADAPT projects (UNIVPM), lines used as **controls to recognise pathogens resistances European elite snap bean varieties**.
(for some materials RNAseq, WGS, GBS is available)
SNAP BEAN PANEL (SBP)
Includes ~300 lines: bush type beans from BEAN_ADAPT Pv_core 1 (~ 60 snap bean lines genotyped by GBS and/ or WGS data from BEAN_ADAPT) and European and worldwide snap bean lines/ elite varieties.
TUM (160 $F_{2:6}$ lines)
Recombinant Inbreed Line (RIL) population derived from the cross between Musica and TU. Musica is a snap bean cultivar with very large, flat and green pod, and TU, an anthracnose differential cultivar with shoert, rounded and purple pod. TU has the Co-5 resistance gene to anthracnose.

The current paper presents a briefly overwieu of P11_VRDS Bacau developed investigations aimed to accomplish BREEDING SET of materials for bean species.

ILs_2018 and ILs_2019 field trials were established in certified organic fields owned by VRDS Bacau, located in NE part of Romania. Simlar experimental fields were established in two other locations in Italy and Spain.

The Romanian area is characterized by the following climate conditions: 550 average annual rainfall (mm), which are not uniformly distributed during the year, and average daily temperature of 9 (°C) across the entire year. Total precipitation and average temperatures data during the vegetation period of the first year of investigation are reported in Figure 2 a and b. The experimental field was characterized by a medium alluvial evolved soil, with clay-sandy texture, and a slightly acid pH (6.2-6.5). Humus content is 2.5-2.7%.

For ILs_2018 trial the collection or field experiment included 300 ILs not replicated, 5 advanced breeding ILs and 2 parental lines, that were all replicated three time in the field. The entire material was provided by the BEAN crop coordinator group (UNIVPM). For investigation of ILs collection, 10 seeds per each line were sown, for a total of 321 plots.

ILs_2019 field trial included also 5 advanced breeding ILs, 2 parental lines, and 300 ILs. The criteria that we adopted for performing the phenotypic selection are described in the "*characterization data*" bellow.

Synthesis of applied cultural practices:

- The material was sown in alveolus in green house conditions. This is a common practice during experiments in order to avoid loses of material (available of limited quantities) during the rainy and cold condition during germination process.
- The seedling was planted in rows at 70 cm, the distance between plants 25 cm. 14 ILs/ row (2018) and 15 ILs/ row (2019)
- No mulch was applied, manually weeding was applied and drop irrigation ensured.
- No supplementary fertilization was applied during 2018.
- Organic fertilization with 1t ha^{-1} applied in 2019.
- Hailstone become more frequent in the last decade in P11 area and for this reason a net protection against hailstone was establish on the experimental fields. No hailstone was registered during both experimental years. No accidents related strong winds were registered during experimental period.
- 4 treatments with Konflic 0.3% and Neemex 0.3% to control aphids from experience.
- Coating treatments with copper products (0.5% Bordeaux mixture)

A common phenotyping kit was used; this protocol reports the list and description of phenotypic data which were recorded; in particular, two categories of data were established, named "Priority traits 1", and "Priority traits 2". "Priority traits 1" included a set of traits, for which phenotyping was mandatory for all the three experiments developed in three different locations Romania, Spain, Italy. "Priority traits 2" included traits for which phenotyping was optionally performed by different partners involved in field trials. Moreover, each partner was encouraged to perform phenotyping for additional traits consider as useful to be recorded, also in relation to euqipments/ tools which can be available at each location.

<u>Priority 1 traits</u>

The "priority 1" set includes the recording of:

1. **Sowing date in plateau** (only if transplant is planned);
2. **Sowing date in field**, date of transplant or sowing date in field in the case transplant is not planned);

<u>Phenological traits</u>

3. **Days to emergence**, number of days after sowing at which 50% of seeds have emerged (only if transplant is not planned);
4. **Days to beginning of flowering**, number of days between sowing date and the appearance of the first flower in the parcel;
5. **Days to flowering**, counting the number of days after sowing at which half of the plants of the plot carried at least one opened flower (*i.e.*, with visible flower banner);
6. **Days to maximum flowering**, number of days required for observing all the plants of the same plot with at least one opened flower;
7. **Days to the end of flowering**, date after sowing at which all plants show no flowers;
8. **Days to harvest as snap bean**, number of days needed for reaching the pod developmental stage R8 (*i.e.,* pod filling);
9. **Days to maturity**, number of days at which the first pod started to discolor and dry in half of the plants of the same plot;

Growth habit
10. **Growth habit**, determinate/indeterminate habit;
11. **Growth habit type**, types of growth habit following Debouck (1986); type I, II, III, and IV correspond to determinate bush, indeterminate bush upright, indeterminate prostrate, and indeterminate climbing, respectively (Figure 1).

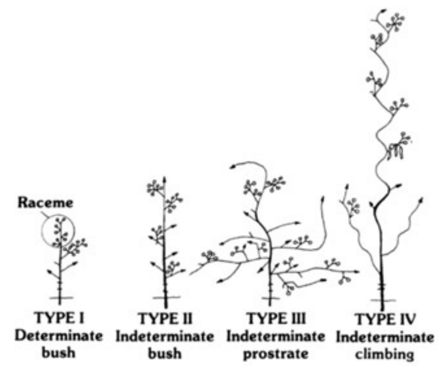

Fig. 1 Growth habit type, after Debouck (1986)

Architectural traits
12. **Pod distribution**, % of pods on the lower, middle and upper thirds of the plant at maturity;
13. **Canopy height** (cm), the distance between the soil surface and the top of canopy at blooming stage;
14. **Stem diameter** (cm), measured right above the soil surface on at least three plants at maturity;
Yield components
Symptomes of diseases
15. **Susceptibility and symptoms of diseases** were recorded during the phenotyping for the main common bean fungus, bacterial, viruses and pest hosts.

Moreover, the type II growth habit can be further classified into type IIa (completely upright) and type IIb (inclined upright); the type III growth habit is further classified into type IIIa (totally prostrated) and type IIIb (main stem and branches can show climbing ability, although they are not very well developed), while the type IV class further comprises the type IVa (pods are uniformly distributed in the plant) and the type IVb (pods are mainly concentrated in the upper part of the plant) (Debouck, 1986). In addition to the four main types, we also considered the growth habit type V (*i.e.*, determinate climbing).

RESULTS AND DISCUSSIONS

All colleted field trial data were stored using Excel data spreadsheet files. Phenotypic data collected during the three field experiments were all reported on a unique Excel (xls.) file, shared among field partners and uploaded in the BRESOV Project Angel.

Briefly, beside the Priority 1 traits, P11 investigated some Priority 2 traits: plant height (in cm; mean of 3 plants for each line), leaf shape (fourth leaf counted from the base to the top), seed brilliance, seed shape longitudinal. As additional traits, P11 investigated length, width and color of pods. The first two traits were included in the "Priority 1" set for ILs_ 2019 investigation. Dry weight of plants was also investigated. All data are under the subject of integration and interpretation comparatively, according to results obtained in three different locations.

Germination - rapid and uniform seed germination and seedling emergence under diverse environmental conditions is a desirable characteristic for crops (De Ron et al., 2016).

Flowering - in most of the vegetable crops, early flowering and maturing genotypes were considered preferable. The influence of genotypes, day length and temperature on flower initiation and fruiting of bean registered values similar to those that also been reported by other researchers, Neupane et al. (2008).

Earliness is a desirable trait in NE part of Romania, due to the climate tendency to decrease the length of period with proper conditions which can affect in future vegetable cultivation in open field condition.

Presence of pests and pathogens related symptoms were investigated during ILs_2018 and ILs_2019 field trials, considering for the main pathogens of the bean crop in Romania: *Bean common mosaic virus BCMV, Bean yellow mosaic virus,* Common blight - *Xanthomonas campestris pv. phaseoli*, Fuscous blight - *Xanthomonas phaseoli* var. *fuscans*, Halo blight of bean - *Pseudomonas syringae pv. phaseolicola,* Bacterial villt of beans - *Corynebacterium flaccumfaciens,* Anthracnose - *Colletotrichum lindemuthianum*, Rust - *Uromyces phaseoli* var. *typica*, Rhizoctonia root rot - *Rhizoctonia solani*, Fusarium root rot - *Fusarium solani* f. sp. *phaseoli,* White mold - *Sclerotinia sclerotiorum,* Gray mold - *Botrytis cinerea.*

With regard to the pests, black bean aphid - *Aphis fabae*, bean weevil - *Acanthoscelides obtectus* Say., seedcorn maggot - *Delia platura* Meig., European mole cricket - *Gryllotalpa gryllotalpa,* and slug - *Deroceras agreste,* were considered for monitoring.

The observations were accomplished every 10 days in May–August period. The attack estimation was determined using the following indicators: Frequency of attack (F%); Intensity of attack (I%); Degree of attack (DA%). Traits related to yield potential were investigated: no plants with pods per plot (count), weight of dry pods per plot (g), weight of ten dry pods per plot (g), no seeds in ten pods (count), no seeds in ten pods note, weight of dry seeds in ten pods (g), weight of 25 seeds (g), weight of dry seeds per plot (g). Entire evaluation allowed a selection of the best 100 ILs for each location. ANOVA Analysis with software JMP ver. 8.0 for all the quantitative traits, comparing selected ILs and non-selected ILs for each ILs_2018 field trial, separately was accomplished by crop coordinator based on analyses of all collected data (Figures 2a and 2b).

Table 2. Bean collections investigated dring 2018 and 2019

Biological material		
2018	321 plots	300 ILs not replicated
		5 advanced breeding ILs + 2 parental lines - replicated (3 replicates)
2019	339 plots	300 lines (3*100 best lines of 2018 from each locality)
		7 common checks used in also in 2018 field experiments, consists in 5 advanced breeding ILs + 2 parental lines – (3 replicates)
		the best 3 lines in each locality, (3 replicates)

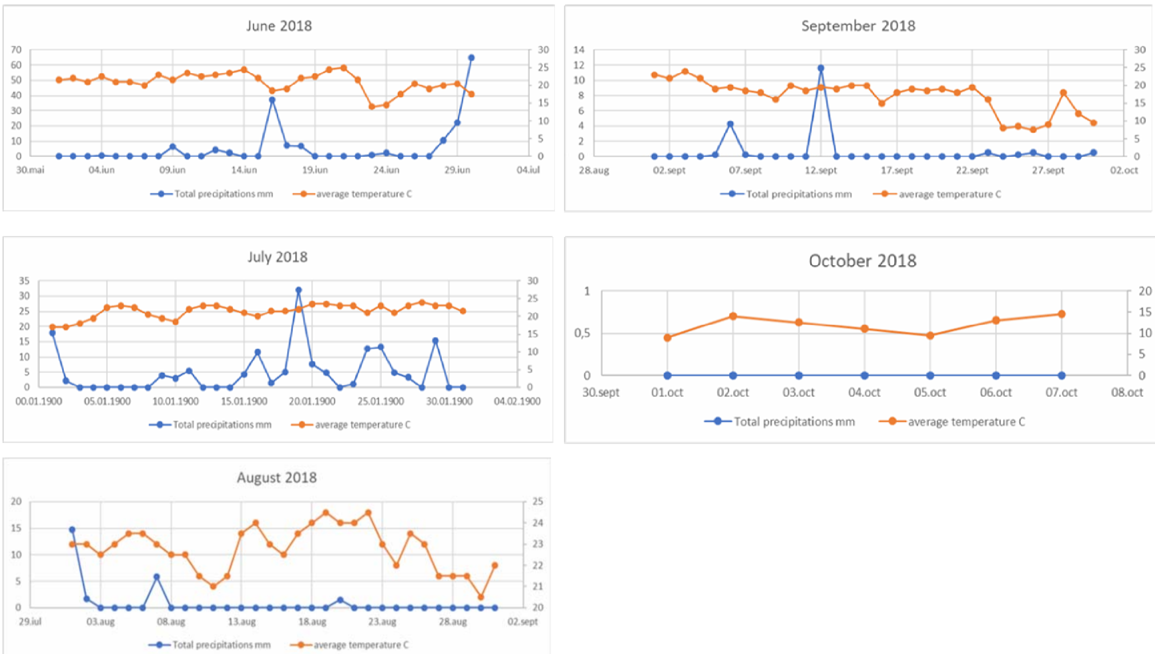

Fig. 2 a Total precipitation and average temperatures during the bean growing season (ILs_2018).

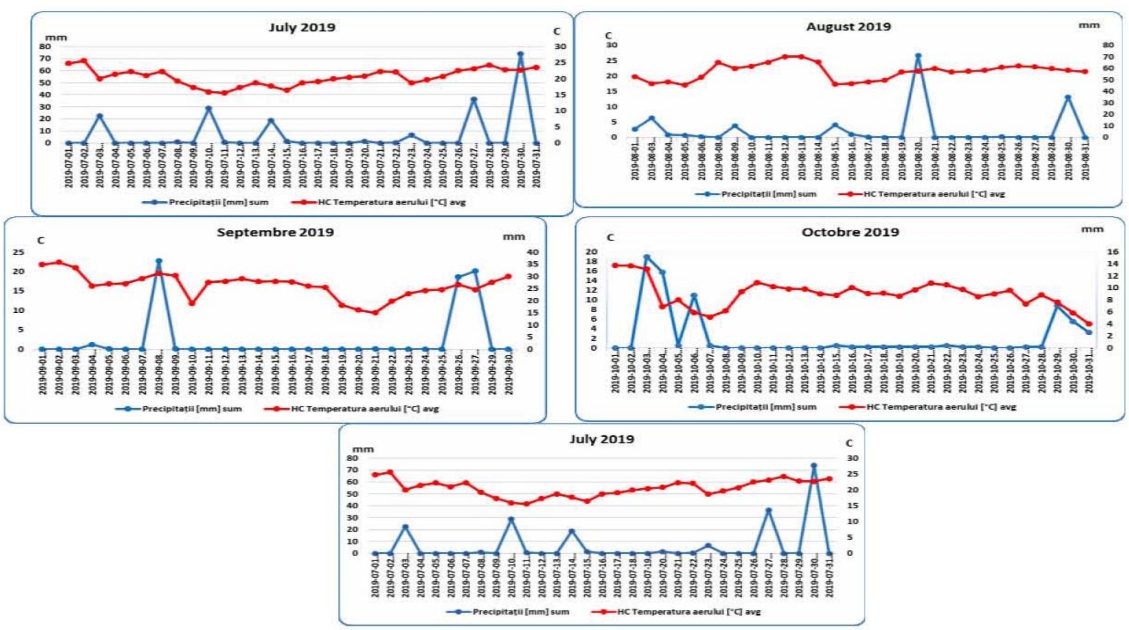

Fig. 2 b Total precipitation and average temperatures during the bean growing season (ILs_2019).

CONCLUSIONS

As a general consideration:

- most lines showed a good plant phenotype: erect and concentrated production was observed in the R8 stage (pod filling; Schoonhoven and Pastor Corrales, 1987). All lines had determinate growth habit, except one IL in P11, which were assigned, respectively, to an intermediate class (both determinate and indeterminate plants in the same plot), and to the indeterminate class;
- all data collected using common template, were compiled in a common data file together with data collected by the other two partners (Italy and Spain) involved in tasks; the file was uploaded on Project angel by crop group coordinator UNIVPM; the task is ongoing for field investigation one more year.
- all data were integrated, at level of crop group coordinator, in order to develop BREEDING SET (BS). A huge volume of information and data were collected with the other two partners results in order to fulfill (D3.2) - BREEDING SET of assembled selected and breeding materials, characterized and evaluated for key morphological and agronomic traits using new crop specific phenotyping kits by partners specialized in ben crop.
- precipitations were not uniformly distributed, cold weather was frequently registered especially at the beginning of vegetation period and drought occurred in flowering and pod set period.

According to the GA conditions, all data will be compilated, integrated and commonly shared in publications.

ABSTRACT

The general objective is to develop high-quality, resilient cultivars for snap bean adapted to organic agriculture based on a set of materials identified in early stages of the project (BREEDING SET) based on already existing available information. In this way, we focussed on exploiting available genetic diversity from the newly assembled BRESOV repository plus selected plant materials developed. Selection and breeding schemes, including complementary hybridization, backcross breeding and pyramiding breeding, performed, will be used to select and develop new materials specifically adapted to organic breeding. Phenotyping kits developed for bean crop were used for selection of complex traits related to resilience under organic conditions, specifically tolerance or resistance to crop-specific relevant abiotic and/or biotic stresses. The activity will allow us to select germplasm in the BRESOV repository that are resilient, and adapted to organic agriculture, to identify sources of tolerance or resistance to prevalent pests and diseases under organic conditions, to breed new elite resilient materials for organic agriculture, and to evaluate quality traits for selecting high-added value cultivars and materials for organic agriculture.

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