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PRE-BREEDING FOR DIVERSIFICATION OF PRIMARY GENE POOL IN ORDER TO ENHANCE THE GENETIC PEPPER RESOURCES

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Abstract

Exploitation of new and diverse sources of variation is needed for the genetic enhancement of Capsicum annuum L. *species. Variety is an important factor of production and has to be in accordance with consumer needs. This study represents a screening of physiology and biochemistry of pepper, particular the Romanian cultivars. The investigated parameters were selected because of their importance in pepper quality, with the final purpose to identify the most valuable resources to be used in breeding as staring material. In this purpose, the study focused on the phenological observation, biometrical measurements and also physiological processes that occur in fruits during their growth and development, which included the following characteristics: total dry content and water content; content in soluble glucides and titratable acidity, content in β carotene and anthocians, content in ascorbic acid, all parameters investigated are related with fruit quality. The most valuable cultivars, regarding nutritional quality were: Creola, characterised by 7.90% content in dry substance, TA 0.343 mg g^{-1} malic acid, AA 200.4 mg g^{-1}, and carotenes 23.452 mg g^{-1}; Lider, 7.80 % content in dry substance, TA 0.344 mg g^{-1} malic acid, AA 200.2 mg g^{-1} and carotenes 23.47 mg g^{-1}; Cornel content in dry substance 7.40%, TA 0.331 mg g^{-1}, AA 199.2 mg $g^{-1}$and carotenes 23.0 mg g^{-1}.*

***Key words**: biometrical measurements, phenological observation, physiological investigations.*

INTRODUCTION

The pepper fruits are very tasty, healthy owing to their content of biologically active chemicals with antioxidant properties. The vegetable is an abundant source of vitamin C (Buczkowska and Najda, 2002). Physiologically ripe fruits are abundant in carotenoid pigments.

Moreover, pepper is an important source of minerals for humans (Bubicz et al., 1999). Consumers have high demands in terms of color, shape, size and taste of fruit and the producer must meet these requirements, making extra productivity, precocity and resistance to pathogens. There is the need to create better varieties with higher yield and quality to satisfy a growing demand (Pérez-Grajales et al., 2009).

The narrow genetic bases of cultivars coupled with low utilization of genetic resources are the major factors limiting production and productivity globally. Wild relatives with enhanced levels of resistance/tolerance to multiple stresses provide important sources of genetic diversity for pepper improvement. Otherwise, the local population or the developed cultivars can represent valuable sources for pepper improvement in terms of nutritional quality.

Pre-breeding provides a unique opportunity, through the introgression of desirable genes from wild germplasm or into genetic backgrounds readily used by the breeders with minimum linkage drag, to overcome this. Pre-breeding activities using promising landraces, wild relatives, and popular cultivars have been initiated, in a diverse range of programs.

Demand for pepper richer in compounds like ascorbic acid, capsaicin, beta-carotene and lycopene is increasing especially because of their demonstrated antioxidant potential.

The future production of cultivated pepper depends on improving their genetics and developing new superior cultivars with traits such as nutritional quality, disease resistance, and higher yield potential. Reported levels of phytochemical variation is due in large part to various environmental conditions (abiotic and biotic stresses) acting on plants during their growth and development (Leskovar et al., 2009). None the less, continual selection of material containing higher levels of these phytochemicals is a valuable component of a breeder‘s program and will undoubtedly result in creation of improved germplasm consumers can eat to benefit their well-being (Crosby et al., 2007).

Significant variation in phytochemical expression within pepper fruit tissue is dependent upon several factors. Genotypic, as well as, environmental differences have both contributed to material of variable phenotypic expression (Draghici, 2014). The ultimate goal of pepper breeders is, therefore, to use knowledge and apply it in a special manner to exploit more effectively match the best genotype with its optimum environment to achieve the most desirable output.

MATERIALS AND METHODS

The experiments were conducted at the Vegetable Research and Development Station Bacau.

The investigated genotypes were grown in open field, natural conditions.

The biological material was represented by twelve Romanian cultivars, as follows: ‘Splendid’, ‘Madalin’, ‘Meteorit’, ‘Cornel’, ‘Lider’, ‘Creola’, ‘Granat’, ‘Timpuriu de Bucureşti’, ‘Titan’, ‘Rubin’, ‘Superb’, ‘Globus’, all cultivated in similar experimental condition.

All fruits harvested for investigations were selected at an appropriate maturity stage and size, and were healthy and turgid.

The phenological observations and the biometrical measurements were accomplished in the experimental parcels, and involved: colour at physiological maturity, fruit’s length (cm), fruit’s diameter (cm), ratio length/ diameter, number of lobs, fruit’s weight (g), number of fruits /plant, pulp’s width (mm).

Fruit measurements were conducted on fruits to gain insight into their potential variation.

The physiological changes monitored were: the content in total dry matter, water and minerals, soluble dry matter, titratable acidity, β carotene, anthocians, ascorbic acid, glucides, all related with nutritional quality of pepper.

Maturated peppers have been collected in the same week of ripening and were chemically analyzed.

The determination of total dry matter substance was carried out by weighing the fresh vegetal material, drying it for 24 hours at 105°C, cooling it, and then weighing again the dry vegetal material. The obtained results were expressed in percentage. The difference till 100% represents the water content.

The content in mineral elements was determined by tissue incineration at 560 ^{o}C temperature and the results were expressed in percentage.

The soluble dry matter content was determined using a refractometer method and the results were expressed as a percentage.

Titratable carotene content was extracted in petrol ether and determined using a spectrometer at λ=415 nm. The content of β carotene was expressed in mg 100^{-1} g. The anthocianic pigments were extracted in methyl alcohol + 1 % HCl and spectrophotometrically determined at 540 nm wave length.

Ascorbic acid was extracted in oxalic acid 1 %, and determined with a Nexus spectrometer (FT-IR). The quantity of ascorbic acid was expressed in mg 100g $^{-1}$.The content in soluble sugar was determined by Fehling method.

RESULTS AND DISCUSSIONS

The breeding program of pepper, founded by Cardi in 1997, has managed to get results in three directions; among those is the use of local landraces. A selection for stability has permitted creation of valuable cultivars from local landraces (Herman, 2005).

Our germplasm study has a multiple approach (1) the screening of phenological and morphological aspects in order to detect the most valuable resources according to the market request: pulp’s width, number of lobs, colour, shape - ratio length/ diameter, fruit’s weight; (2) investigation of internal quality: total dry matter, water and minerals, soluble dry matter, titratable acidity, β carotene, anthocians, ascorbic acid, glucides, in order to distinguish the most favorable germplasm for potential release in the future.

The main fruit's characteristics investigated are presented in table 1 - round pepper (12 genotypes). The most obvious trait of interest to breeders and growers is uniformity. Because pepper are a self-pollinating crop, this has been accomplished by inbreeding peppers, while selecting for important shape, flavor, appearance and yield traits by breeders throughout the world.

Table 1. Phenological observations and biometrical measurements – round pepper

Variety	Fruit			
	Length (cm)-	Diameter (cm)	Ratio L/D	No of lobs
Splendid	7.2 ± 0.030	8.6 ± 0.02	0.83 ± 0.03	3.2 ± 0.10
Madalin	7.8 ± 0.021	7.9± 0.01	0.98 ± 0.06	3.8 ± 0.10
Meteorit	7.1 ± 0.010	7.9± 0.02	0.89 ± 0.18	2.8 ± 0.04
Cornel	7.1 ± 0.040	8.2± 0.01	0.86 ± 0.21	2.9 ±0.05
Lider	7.0 ± 0.031	8.2± 0.02	0.85 ± 0.05	2.9 ± 0.17
Creola	7.9 ± 0.012	8.8± 0.01	0.89 ± 0.02	3.6 ± 0.12
Granat	7.1 ± 0.013	7.6± 0.02	0.93 ± 0.04	2.6 ± 0.9
Globus	5.6 ± 0.012	5.9 ± 0.02	0.94 ± 0.04	2.1 ± 0.14
Titan	7.4 ± 0.011	8.7± 0.02	0.85± 0.02	3.2 ± 0.52
Rubin	5.2 ± 0.020	5.9± 0.03	0.88± 0.01	2.8 ±0.42
Superb	5.9 ± 0.050	6.2± 0.04	0.95 ±0.04	2.7 ± 0.37
Timpuriu de Bucureşti	5.3 ± 0.040	6.0 ± 0.05	0.88 ± 0.05	2.8 ±0.12
Average	6.7	7.5	0.89	2.70
Standard deviation	2.56	1.33	1.6	0.29
LSD 0.05	0.98	0.72	0.85	0.20

Table 2. Phenological investigation at round pepper fruits (weight, pulp's width, number fruits/plant)

Variety	Shape	Colour at physiological maturity	Fruit		
			Weight -g-	Pulp's width -mm-	Number of fruits /plant
Splendid	round - flattened	Red	70.8 ± 2.5	7.8 ± 0.090	9.2 ± 0.75
Madalin	round - flattened	red-carmine	80.6 ± 3.9	9.1 ± 0.047	6.8 ± 0.82
Meteorit	round	dark red	85.7 ±2.23	9.4 ± 0.180	7.3± 0.75
Cornel	round	Red	90.8 ± 2.4	10.2 ± 0.550	8.5 ± 0.80
Lider	round - flattened	red-carmine	75.4 ± 1.55	9.5 ± 0.600	10.3 ± 0.98
Creola	round	shiny red	215 ±4.20	12.9 ±0.190	12.8 ± 1.21
Granat	round	dark red	110 ±1.55	9.8 ±0.500	9.1 ± 1.23
Globus	globular	red-carmine	98 ± 2.20	11.2 ± 0.420	15.2 ± 1.55
Titan	spherical	dark red	180 ± 1.95	11.6 ± 0.370	6.2 ± 1.21
Rubin	round	red	90.5 ±2.45	8.9 ± 0.620	9.4 ± 1.6
Superb	round - flattened	red	97.4 ± 2.33	8.2 ± 0.550	-
Timpuriu de Bucureşti	globular	red	90.9± 1.9	8.8 ± 0.230	11.9 ± 0.66
Average			107.09	9.78	8.89
Standard deviation			85.8	1.02	2.66
LSD 0.05			30.26	0.45	0.98

Some of the most important features related with visual quality of pepper are the external color, weight of fruit, shape and pulp' weight. The tremendous variability regarding shape, color and weight of bell pepper fruits is totally different, being lower in case of round pepper germplasm. In any case, the fruit of round pepper must be fully red to be acceptable for processing and desirable for their decorative color and the flavor they impart to processed food. The consumers prefer a red fruit in a ready-to-eat stage with an attractive appearance, a crisp texture and have a specific flavor.

In our collection, the shape varied from round flattened to round, globular, with an average of ration length / diameter 0.89 (Table 1 and 2). Comparing the length and diameter of investigated genotypes, we observed that eight genotypes registered large fruits with length more than 7 cm and diameter more than 7.6 cm. The smallest fruits regarding length and diameter values were harvested from genotype 'Rubin", 5.2 cm length and 5.9 cm diameter.

The variation of total number of lobs was between 2.1 and 3.8 with an average of 2.7. Table 2 presents the variability of fruit weight, pulp width and number of fruit per plant. The value of fruit weight varied from 70.8 g ('Splendid'), to 180 g ('Titan') and 215 g ('Creola'). The heaviest fruits of 'Creola' genotype, registered the highest value of pulp's width (12.9 mm).

The shape, the size and the fruit's weight are important parameters of yield and quality as follows: fruit's weight cumulated with number of fruits per plant, density, etc. - especially in case of yield potential estimation and fruit's weight, pulp's width, fruit's shape, and fruit's size for establishment of crop use - fresh consumption or as raw material, in food industry.

Figures 1 to 5 presents the results of the analyses focused toward the determination of the chemical composition of 12 genotypes of round pepper. The size and the quality of mature peppers were determined by interaction of genotype x environmental climate. One of the indicators of size and round pepper quality is represented by the accumulation of water and total dry matter.

The total dry matter (TDM) content varied from 7% to 7.9 %. The highest content was registered in fruits of 'Lider" and "Creola" genotypes, 7.9%, respectively 7.4%. One of the factors affecting the production of plant biomass is the concentration of mineral elements.

Regarding mineral accumulation, the total amount of minerals in round pepper fruits varied in limits of 0.09%, from 0.46% at 'Spelendid', 'Madalin', 'Titan', 'Rubin' to 0.55% at 'Globus" genotype (Figure 1).

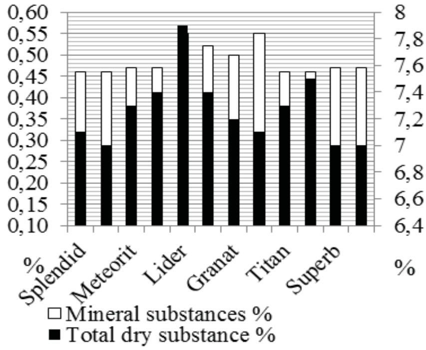

Figure 1. Total dry mater and mineral accumulation

Fruit quality and consumer acceptability in round pepper are strongly related with the pigments concentration, soluble solids content (TSS) titratable acidity (TA) and ascorbic acid (AA) in the ripened fruits. During ripening process, some substances of important nutritional quality, particularly vitamin C and carotenoids are accumulated in large quantities (Navarro et al., 2006).

Ascorbic acid, known as vitamin C, needs to be consumed via food or medicine, as it is not produced in the human organism (Manela-Azulay et al., 2003). The levels of vitamin C are variable and may be affected by maturity, genotype and processing. Ascorbic acid is the least complex vitamin found in plants and is synthesized from glucose or some other simple carbohydrate (Kays, 1991).

According to our study, the fully ripened round pepper fruits have the highest levels of ascorbic acid (AA), the titratable acidity and the total soluble solids also.

The round pepper, totally maturated represents an important source of vitamin C for human consumption, presenting values of higher than 190 mg 100 g^{-1} ('Meteorit'), till 200.4 mg 100 g^{-1} ('Creola'). Ascorbic acid (AA) concentrations were highly variable among the accessions (Figure 2).

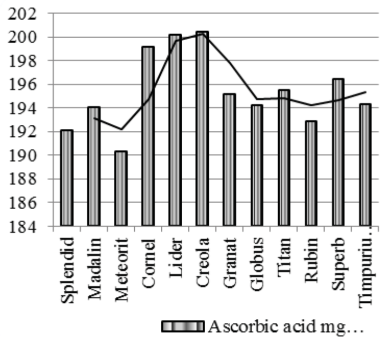

Figure 2. Ascorbic acid in maturated fruits

Our results highlight the antioxidant potential of round pepper species, stronger than those of mango (84 mg 100 g-[1]), guava (67 mg 100 g[-1]) and orange (40 mg 100 g[-1]), Toda Fruta, 2004).

Round pepper is one of the most important sources of vitamin C, and also the β-carotene content, the predecessor of vitamin A. The content of β-carotene was very high in most investigated varieties and fluctuates from 18.96 mg 100g [-1] (‘Meteorit’) to 23.45 and 23.47 mg 100g[-1] at ‘Creola’, respectively ‘Lider’. This compound increased dramatically in mature colored fruit for all lines tested.

Carotenoids concentrations varied tremendously among the germplasm accessions. Six accessions registered low β – carotene content, (under 20 mg 100g[-1]), and other six genotypes were characterized by extremely high (above 20 mg 100g[-1]) total carotenoids at the maturated stage (Figure 3).

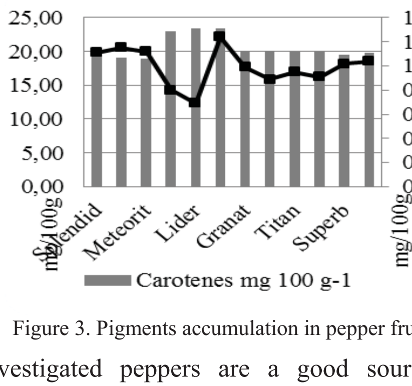

Figure 3. Pigments accumulation in pepper fruits

Investigated peppers are a good source of vitamin C and carotenoids that are important nutritional antioxidants found in the human diet. Many authors reported increase of carotenoids, during development of pepper fruits. In general, ripening of pepper fruits is strongly associated with carotenoids accumulation (Marcus et al., 1999). Anthocians synthesis is also responsible for color of maturated fruits.

The average of the anthocians content was 0.992 mg 100g[-1], with a variation form 0.69 mg 100g[-1] (at ‘Lider’) to 1.24 mg 100g[-1] at ‘Creola’ (Figure 4).

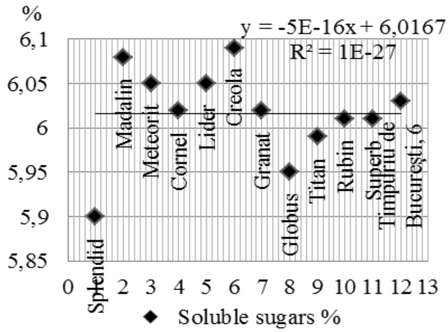

Figure 4. Soluble sugars content

The genotype ‘Creola’ is distinguished by its highest content in anthocians and also in β-carotene. Significant accumulation of soluble sugars during pepper fruit ripening was confirmed by findings of (Howard et al., 2000). In our study the range of soluble sugars varied from 5.90% at fruits of ‘Splendid” cultivar to 6.90 % at ‘Creola’.

Titratable acidity (TA) as well as TSS are commonly measured to give an overview of pepper maturity at harvest, and are used for harvest scheduling. TA indicates the total amount of organic acids. It is already known the fact that titratable acidity (TA) of the round peppers is increased with ripening, while during the ripening process the metabolic reactions increase, increasing the concentration of organic acids involved in the Krebs cycle.

Apart from this, these acids make up the energetic reserves and the metabolic reactions that involve the synthesis of pigments, enzymes and other materials and the degradation of pectins and celluloses, which are essential for the ripening process. Acidity is important for flavor balance. Our study shows a small variation in case of titratable acidity (TA) of investigated genotypes from 0.330 mg g[-1] malic acid (‘Madalin’ and ‘Superb’) to 0.334 mg g[-1] at (‘Lider’) (Fig.5). The total soluble solids

(TSS) increased as ripening of the fruit increased due to the greater degradation or biosynthesis of the polysaccharides and the accumulation of sugars.

The metabolic processes related to the advance of ripening, probably due to disassociation of some molecules and structural enzymes in soluble compounds, directly influence the levels of total soluble solids, where fruits in advanced stages of ripening present the highest levels of soluble solids (Lyon et al., 1992). Regarding the content of total soluble solids (TSS), the variation was form 7.1 % at 'Superb', 'Globus', 'Cornel', and 'Madalin' to 8.3 % at 'Creola'.

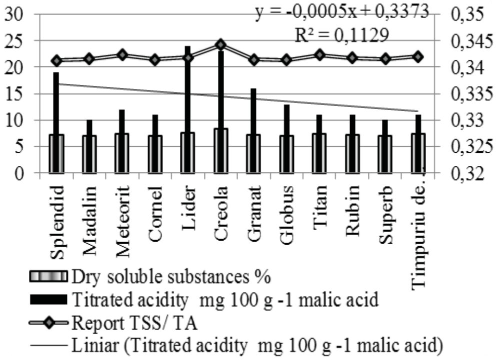

Figure 5. TTS, TA and report between

As TSS, TA and the report between, can be easily and objectively measured, and commonly used as basic quality specifications related to maturity of peppers and therefore suitability for harvest. The report total soluble solids (TSS), and titratable acidity (TA) represents an important qualitative parameter TSS/TA. In our investigation the value of this report varied from 21 to 25 (Figure 5).

CONCLUSIONS

For pepper improvement exists sufficient genetic diversity, especially local germplasm can constitute a gene pole that is still insufficient exploited. However, utilization of these resources in breeding programs is time-consuming and resource demanding. To overcome this, pre-breeding activities should be initiated to generate new genetic variability using promising and valuable genetic material for use by the breeders in pepper improvement programs. Results from this experiment provide evidence that elite pepper materials exist for these characteristics of interest.

This preliminary study can be potentially examined in future investigations and possibly exploited in various breeding methods to maximize their potential superiority as parent material for development of several improved specimens.

Three genotypes present superior qualitative traits: Creola, characterised by 7.90% content in dry substance, TA 0.343 mg g^{-1} malic acid, AA 200.4 mg g^{-1}, and carotenes 23.452 mg g^{-1}; Lider , 7.80 % content in dry substance, TA 0.344 mg g^{-1} malic acid, AA 200.2 mg g^{-1} and carotenes 23.47 mg g^{-1}; Cornel content in dry substance 7.40%, TA 0.331 mg g^{-1}, AA 199.2 mg $g^{-1}$and carotenes 23.0 mg g^{-1}.

Our study highlight the need of enormous efforts needed to evaluate germplasm for traits of economic importance, for identifying potential donors. The success of pepper improvement program depends on the availability of sufficient genetic variability, but this variability must be in conventionally usable form.

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