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Heterosis and combining ability in bell pepper lines with resistance to multiple pathogens[1]

Heterose e capacidade combinatória em linhagens de pimentão com resistência a múltiplos patógenos

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ABSTRACT - The aim of this study was to develop bell pepper hybrids with resistance to multiple pathogens and make inferences in regard to components of heterosis in hybrids obtained from lines presumed to be resistant to *Pepper yellow mosaic virus* (PepYMV), *Phytophthora capsici*, or *Meloidogyne incognita*. The studies were carried out in a greenhouse. We used ten lines, thirty experimental hybrids, and seven controls (Konan-R, Magali-R, Martha-R, Stephany, Mallorca, Magnata Super, and Criollo de Morelos-334). For all experiments, a randomized block design was used with three replications (with plots composed of 16 plants). For evaluation of the reactions to *P. capsici* and to PepYMV, the percentages of asymptomatic plants were considered. For evaluation of the reactions to *M. incognita*, the reproduction index and the reproduction factor of the nematode were calculated. The additive gene effects were important for all the traits evaluated, and for percentage of plants resistant to PepYMV and *P. capsici*, the non-additive effects were also important. The alleles that control resistance to PepYMV, to *P. capsici*, and to *M. incognita* have a degree of dominance near 1, in absolute value, which indicates a favorable situation for obtaining hybrids that accumulate multiple resistance to these pathogens.

Key words: *Capsicum annuum*. Disease resistance. Plant breeding.

RESUMO - O objetivo deste trabalho foi desenvolver híbridos de pimentão com resistência a múltiplos patógenos e inferir sobre os componentes da heterose em híbridos obtidos a partir de linhagens presumivelmente resistentes ao *Pepper yellow mosaic virus* (PepYMV), *Phytophthora capsici* ou *Meloidogyne incognita*. Os trabalhos foram realizados em casa de vegetação. Foram utilizadas dez linhagens, trinta híbridos experimentais, e sete testemunhas comerciais (Konan-R, Magali-R, Martha-R, Stephany, Mallorca, Magnata Super e Criollo de Morelos-334). Em todos os experimentos, utilizou-se o delineamento em blocos casualizados, com três repetições (com parcelas de 16 plantas). Na avaliação das reações a *P. capsici* e ao PepYMV, foram consideradas as percentagens de plantas sem sintomas. Na avaliação das reações a *M. incognita*, foram calculados o índice de reprodução e o fator de reprodução do nematoide. Os efeitos gênicos aditivos foram importantes para todos os caracteres avaliados e, para percentagem de plantas resistentes a PepYMV e *P. capsici*, os efeitos gênicos não-aditivos foram também importantes. Os alelos que controlam resistência a PepYMV, a *P. capsici* e a *M. incognita* possuem grau de dominância próximo de 1, em valor absoluto, o que indica uma situação favorável à obtenção de híbridos com resistência múltipla a estes patógenos.

Palavras-chave: *Capsicum annuum*. Resistência a doenças. Melhoramento de plantas.

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INTRODUCTION

Bell pepper (*Capsicum annuum* L.) is one of the most widespread vegetables and very widely consumed *in natura* in Brazil, and it is among the ten crops of greatest economic importance in the vegetable market. Bell pepper production is predominantly in open fields, concentrated in the states of São Paulo, Minas Gerais and Paraná which together produce an average of 160 thousand tons per year, comprising around 50% of the planted area in Brazil, which is 12,000 hectares (AGRIANUAL, 2016; HENZ *et al*., 2007; MOURA *et al*., 2012). One of the main factors for the increase in planted area and yields is the use of more productive commercial hybrid or cultivars with resistance to a greater number of diseases (NASCIMENTO *et al*., 2007, 2010).

In spite of breakthroughs in improving productive systems, diseases have been major drawbacks for a more expressive increase in yields. Among the main diseases of this crop are those caused by *Phytophthora capsici* Leonian, Pepper yellow mosaic virus (PepYMV), and *Meloidogyne incognita*.

P. capsici brings about blight or root rot, which has shown to be one of this crop's most destructive diseases throughout the world (MCGREGOR *et al*., 2011; NAEGELE; TOMLINSON; HAUSBECK, 2015). This pathogen can infect all parts of the plant, rotting root and its collar, fruit, causing black lesions on stem, brown circular lesions on leaves, and its death (FOSTER; HAUSBECK, 2010; NAEGELE *et al*., 2014). PepYMV occurs naturally in most Brazilian crop producing regions, causing serious losses, and being this crop's major viral disease (INOUE-NAGATA *et al*., 2002; LUCINDA *et al*., 2012; MOURA *et al*., 2011). *M. incognita* decreases fruit yield and it is found in crop fields (GISBERT *et al*., 2013; SÁNCHEZ-SOLANA *et al*., 2016). Conversely, most commercial varieties shown to be resistant to *M. javanica* (PEIXOTO; MALUF; CAMPOS, 1995).

Genetic *Capsicum* genus variability has enabled cultivars to become resistant to these pathogens, which has been a priority in bell pepper breeding programs (CANDOLE; CONNER; JI, 2010; DI DATO *et al*., 2015; FAZARI *et al*., 2012; NOGUEIRA *et al*., 2012). This genetic resistance can be exploited in hybrid combinations, which allow the combination of different desirable traits, both qualitative and quantitative, in a single genotype.

The aim of this study was to develop bell pepper hybrids with resistance to multiple pathogens and make inferences in regard to components of heterosis in hybrids obtained from lines presumed to be resistant to PepYMV, *Phytophthora capsici*, and *Meloidogyne incognita*.

MATERIAL AND METHODS

The studies were carried out in a greenhouse in the experimental area of the company HortiAgro Sementes S.A., in the municipality of Ijaci, MG, Brazil.

The genetic material tested consisted of 47 bell pepper genotypes composed of (a) ten lines (used as parents in obtaining experimental hybrids); (b) thirty experimental hybrids, twenty of which were obtained from crosses of two groups of parents (group I – female parents: 1=PIX-044B-01-01, 2=PIX-044B-13-01, 3=PIX-045B-27-02, 4=PIX-045B-32-03, and 5=PIX-052B-06-01; group II – male parents: 1'=Carolina Wonder, 2'=Charleston Belle, 3'=MYR-29-09-05, and 4'=MYR-29-11-08), and ten were additional hybrids corresponding to the crosses: F1(PIX-044B-01-01 x PIM-13), F1(PIX-044B-13-01 x PIX-052B-06-01), F1(PIX-045B-27-02 x PIX-052B-06-01), F1(PIX-045B-32-03 x PIX-052B-06-01), F1(PIM-13 x MYR-29-09-05), F1(PIM-13 x MYR-29-11-08), F1(Carolina Wonder x MYR-29-09-05), F1(Carolina Wonder x MYR-29-11-08), F1(Charleston Belle x MYR-29-09-05), and F1(Charleston Belle x MYR-29-11-08); and (c) six commercial controls (Konan-R, Magali-R, Martha-R, Stephany, Mallorca, Magnata Super), as well as the accession Criollo de Morelos-334). In all the experiments, Magnata Super and Criollo de Morelos-334 were used as controls for susceptibilities and resistance to the pathogens (*P. capsici*, PepYMV and *M. incognita*), respectively. The parent lines and their characteristics are described in Table 1. In diallel analysis, only data from the 20 hybrids obtained from crosses of the parents of group I with those of group II were used.

The lines PIX-044B-01-01, PIX-044B-13-01, PIX-045B-27-02, PIX-045B-32-03, and PIX-052B-06-01 came from breeding programs conducted at the company HortiAgro Sementes S.A., and given their genealogy and the selection processes used, they are presumably resistant to both PepYMV and *P. capsici*, but susceptible to *M. incognita*. PIM-013 is an elite line from HortiAgro with resistance to *P. capsici*, and susceptibility to both PepYMV and *M. incognita.* MYR-29-09-05 and MYR-29-11-08 are HortiAgro lines selected for greater uniformity of fruit shape by successive self-pollination from the open pollination population MYR-29, considered resistant to PepYMV (NASCIMENTO *et al*., 2007). MYR-29-09-05 and MYR-29-11-08 are known to be resistant to PepYMV, but are, a *priori,* presumed susceptible to both *P. capsici* and *M. incognita*, although this still needs to be confirmed. Carolina Wonder and Charleston Belle are lines obtained by the U.S. Vegetable Laboratory, USDA/ ARS, Charleston, SC, USA; they are homozygous for the N gene, which confers resistance to the nematode *M. incognita* (FERY; DUKES; THIES, 1998). Their reactions

Table 1 - Description of parental lines in regard to reaction to the pathogens PepYMV, *P. capsici*, and *M.incognita* in bell pepper

Parental lines	Reaction[1]		
	PepYMV	P. capsici	M. incognita
		Group I	
PIX-044B-01-01	R (?)	R (?)	S (?)
PIX-044B-13-01	R (?)	R (?)	S (?)
PIX-045B-27-02	R (?)	R (?)	S (?)
PIX-045B-32-03	R (?)	R (?)	S (?)
PIX-052B-06-01	R (?)	R (?)	S (?)
		Group II	
CarolinaWonder	S (?)	S (?)	R
Charleston Belle	S (?)	S (?)	R
MYR-29-09-05	R	S (?)	S (?)
MYR-29-11-08	R	S (?)	S (?)
Additional			
PIM-13	S	R	S

[1] S = Susceptibility; R = Resistance; (?) = indicates presumed resistance (R) or susceptibility (S) based on genealogy and/or on previously obtained information

to PepYMV and to *P. capsici* are presumed to be toward susceptibility, although they have not been described by the authors and, therefore, require confirmation.

The reactions of the bell pepper genotypes to PepYMV, *P. capsici*, and *M. incognita* were evaluated in experiments carried out independently.

In all of the three experiments, the treatments were sown in 128-cell polystyrene trays containing Topstrato® commercial substrate. In each cell, two seeds were sown and, after germination, the plants were thinned, leaving only one plant per cell. Randomized block experimental designs were used, with three replications. The number of plants per plot was 16. Before and after the inoculations, the trays were kept in a greenhouse.

Experiment 1

In the experiment for evaluation of reactions to PepYMV, a potyvirus isolate used, sorologically characterized as PepYMV, provided by the company Sakata Sudamerica and obtained in the region of Lins, SP, coming from naturally infected bell pepper plants with systemic symptoms. To maintain the virus isolate, *Nicotiana tabacum* plants (TNN) and Ikeda cultivar bell pepper plants (susceptible to PepYMV), infected with PepYMV were kept in both silica-gel desiccators and in liquid nitrogen at the controlled temperature of -80 ºC (ultrafreezer). Inoculum for later use in screening for resistance was replicated in *N. tabacum* indicator plants (TNN) kept in greenhouses with screens; the plants were substituted at intervals of around two months. For inoculation in sweet pepper, tobacco leaves infected with PepYMV, used as the source of inoculum, were macerated in 0.01 M phosphate buffer, pH 7.0. After that, the sweet pepper plants to be tested were sprinkled with carborundum (400 mesh) and then the plant extract solution was applied on the leaves. After inoculation, the plants were watered and kept in greenhouses. Two inoculations were performed to avoid possible escapes: the first when the plants achieved the first fully-expanded definitive leaf stage, and the second, seven days after the first. Evaluations were made weekly, from the 15th to the 40th day after the first inoculation, for a total of five evaluations; the evaluations performed on the 40th day were taken as definitive evaluations. Scores were attributed to each plant, ranging from 1 to 5, according to the scale of Nascimento *et al*. (2007), the scores representing: 1- no symptoms; 2- lightened color between leaf veins; 3- light mosaic; 4- highly developed mosaic, without leaf deformation; 5- yellow mosaic, blister-like, with leaf deformation. Plants that had a score of 1 in the evaluation on the 40th day were considered asymptomatic.

Experiment 2

In evaluation of the reactions of resistance to *Phytophthora capsici*, the isolates of this pathogen, Pc11 and Pc31, provided by the company Sakata Sudamerica/Agroflora, Bragança Paulista, SP, Brazil (originally collected in the region of Bernardino de Campos, SP, and Santa Cruz do Rio Pardo,

SP, respectively) were kept in test tubes containing PDA (potato dextrose agar) medium and stored in a Biochemical Oxygen Demand (BOD) chamber. The media containing the isolates were once more cut in small pieces and then placed on top of new Petri dishes containing PDA, where they remained for 4 to 5 days in a BOD chamber at a temperature of 27 ºC. For sporangium production, the media containing the isolates were once more cut in small pieces and placed on 9-cm diameter Petri dishes containing a medium composed of tomato juice/ agar (200 ml of Superbom® tomato juice, 3 g of calcium carbonate, 17 g of agar, and 800 mL of distilled water) at 28 ºC, under continuous light for seven days. After that, 10 – 15 mL of distilled water was added per dish, and light scraping was performed with a Drigalski spatula to detach the sporangia. To release the zoospores, the sporangium suspension was left for an hour at room temperature. The suspension was then filtered through a double gauze layer, and an aliquot of the filtered material was removed to count the number of zoospores in a Neubauer chamber. To do so, the suspension was shaken in a Vortex for one minute to stimulate encysting of the zoospores. After counting was performed and dilution was established at the desired concentration (10^4 zoospores/mL), the zoospore suspension was used immediately. Inoculations were made on seedlings, obtained as previously described and kept in polysterene trays. An inoculum concentration of 10^4 zoospores/mL was used, applying 5 mL of the suspension in each tray cell near the root collar of the plants at 40 days after germination. Evaluations were made as of the third day after inoculation, extending to the 15 th day. Scores ranging from 1 to 3 were attributed to each plant, according to the scale of Nascimento *et al*. (2007), the scores representing: 1- without symptoms; 2- necrosis and wilting; 3- without leaves and dry. Plants with a score of 1 in evaluation on the 15 th day were considered asymptomatic.

Experiment 3

In evaluation of reactions to *M. incognita*, the reproduction index and the reproduction factor of the nematode were evaluated. A known isolate of *Meloidogyne incognita* was used as a source of inoculum, which was previously multiplied and maintained on tomato plants - *Solanum lycopersicum* (= *Lycopersicon esculentum*), cultivar Santa Clara. Extraction of nematode eggs was performed according to the method of Hussey and Barker (1973), modified by Bonetti and Ferraz (1981). Tomato roots containing root knots were cut in pieces of around 0.5 cm length and ground in a blender for 40 seconds with sodium hypochlorite solution at 0.5%. After that, the solution containing the eggs was run through a sieve with a 0.074 mm mesh on top of a sieve with a 0.028 mm mesh; the eggs were then thoroughly washed under running water. The nematode eggs, taken from the sieve with the smaller mesh, were collected and quantified in a stereomicroscope. The substrate was infested 15 days after seed germination using an automatic syringe for veterinary use. An aliquot of the solution containing 2,000 nematodes eggs (initial population) per plant was used for infestation of the substrate in the trays. The viability of the inoculum was quantified by means of hatching chambers. As the inoculum used had viability of 60.4%, the number of viable eggs inoculated on each plant was therefore 1,208. The seedlings were watered daily up to the time of evaluations, which began at 75 days of plant age (60 days after inoculation), when the root system of each plant was cut with a scissors and ground in a blender according to the technique of Hussey and Barker (1973), modified by Bonetti and Ferraz (1981). This was followed by counting the eggs of the final population, using a Peters slide and a stereomicroscope. Finally, the number of eggs per fresh root mass was calculated.

The tomato cultivar TOM-584 was used as a susceptible standard control for comparison with nematode reproduction on the bell pepper seedlings. The reproduction index was calculated in the following manner: (number of eggs per gram of root/mean number of eggs per gram of root of the TOM-584 plants) x 100. The reproduction factor was calculated in the following manner: final population/initial population of viable eggs.

The genetic-statistical analyses for all the traits evaluated consisted of analyses of variance, with breakdown of degrees of freedom, according to the partial diallel model of Miranda Filho and Geraldi (1984), through Genes software (CRUZ, 2013).

RESULTS AND DISCUSSION

PepYMV

Analysis of variance showed significant differences among the treatments for percentage of plants asymptomatic to Pepper yellow mosaic virus (PepYMV) (Table 2). In diallel analysis, both the variety effects of groups I and II and effects of average heterosis ($\overline{h}$), varietal heterosis (h_i, h_j), and specific heterosis (S_{ij}) were significant, indicating the existence of additive gene effects and that significant heterosis is also manifested in their crosses (Table 2).

The estimates of general combining abilities (GCAs) ranged from -5.46 to 7.04 (amplitude of 12.5) among the lines of group I, and from -9.85 to 7.05 (amplitude of 16.9) among the lines of group II (Table 3). In regard to the average (μ= 72.62), the amplitudes of the additive effects (GCA) for the two groups of lines represented 17.21% and 23.27%, respectively, indicating that the lines of group II are more divergent among themselves in regard to the percentage of asymptomatic

Table 2 - Summary of analyses of variance for percentage of plants asymptomatic to PepYMV, *Phytophthora capsici*, reproduction index (RI), and reproduction factor (RF) of *Meloidogyne incognita* in bell pepper

Source of variation	DF	Mean Square			
		PepYMV	Phytophthora capsici	Reproduction Index (RI)	Reproduction Factor (RF)
Blocks	2	85.937	102.246	1640.36	150.397
Treatments	28	2077.487 **	2451.634 **	46246.077 **	2215.883**
Lines of group I vs group II	1	11334.146**	41669.190**	296991.606**	11286.849**
Among lines of group I (vi)	4	577.670**	522.249**	12242.546**	438.293**
Among lines of group II (vj)	3	8172.877**	792.488**	214981.734**	12179.491**
Heterosis	20	1000.309**	1125.505**	15199.158**	623.312**
Average heterosis (Ħ)	1	8003.729**	15467.164 **	234021.842**	7023.348**
Varietal heterosis of group I (hi)	4	154.806*	376.416**	6565.625*	183.577^{ns}
Varietal heterosis of group II (hj)	3	2899.214**	792.371**	8191.634**	1022.849**
Specific heterosis (Sij)	12	223.799**	263.346**	1592.159^{ns}	136.670^{ns}
Mean error	56	41.31	72.08	1686.58	125.24
Mean		89.88	75.53	96.76	22.73
C.V. %		7.15	11.24	42.44	49.22

^{ns}, **,*: not significant, and significant at 1% and 5% probability by the F test, respectively

plants to the PepYMV than the lines of group I. The non-additive effects, S_{ij} (that represent specific combining ability [SCA] or, in a similar manner, specific heterosis), for their part, ranged from -15.74 to 9.84 (total amplitude of 25.58) (Table 3).

This value indicates an important contribution of the non-additive effects, whose amplitude represents around 35% of the average (μ= 72.62) (Table 4), in relation to the additive effects of GCA.

In the cases in which both the parents of group I (parents 2, 3, and 4) and of group II (parents 3' and 4') exhibited 100% asymptomatic plants (Table 5), the heterosis relative to the average of the parents (HRAP%) was 0% (Table 4), a consequence of the absence of genetic divergence among the parents for resistance to PepYMV. Conversely, in crosses involving the same parents 2, 3, and 4 of group I with the lines (parents 1', 2') susceptible to PepYMV, the estimates of heterosis ranged from values near 50% to values near 99%, always in the direction of greater percentage of asymptomatic plants, which indicates that resistance is controlled by dominant alleles whose mean degree of dominance tends to 1. The existence of two lines (1 and 5) in group I not totally fixed for the trait of resistance to PepYMV (Table 5) results in values of heterosis in the hybrids discrepant from the previous bands, especially in the case of parent 1, in which the percentage of asymptomatic plants is significantly less than 100%.

P. capsici

For resistance to *P. capsici*, analysis of variance (Table 2) showed that the most relevant source of variation was the contrast between the lines of group I (1, 2, 3, 4, 5) and those of group II (1', 2', 3', 4'), reflecting a considerable difference between the parents of group I (resistant) compared to those of group II (Table 5). Both the varietal effects (v_i, v_j) and the components of heterosis ($\overline{h}$, h_i, h_j, S_{ij}) were significant (Table 2), indicating the importance of both the additive effects and the non-additive effects in expression of the trait.

The estimates of general combining ability (GCA) ranged from -9.06 to 11.25 (with an amplitude of 20.31) for g_i, and from -7.81 to 7.19 (with an amplitude of 15.00) for g_j (Table 3). In comparison to the average (μ= 57.70) of plants resistant to *P. capsici*, these values represent 35.19% and 25.99%, respectively, which is quite considerable, thereby reflecting the significance of the effects of the components that express the GCAs in analysis of variance (Table 2). High total amplitude was also observed for estimates of specific combining ability (SCA), which ranged from -14.37 to 13.54, i.e. amplitude of 27.91 (Table 3). This amplitude represented 48.37% of the value of the average. These results reinforce the conclusion of the ANOVA (Table 2) that both the additive effects and the non-additive effects contributed to expression of the trait.

All the hybrids tested in the diallel represented combinations between the lines resistant to *P. capsici* (of group I) with less resistant or susceptible lines (of group II). In combinations of the lines of group I (1, 2, 3, 4, 5) with the lines that proved to be more susceptible from group II (3', 4'), the estimates of heterosis in relation to the average of the parents (HRAP%) ranged from +28.80% to +90.76% (Table 4), indicating incomplete dominance of the alleles that control the biggest percentage of asymptomatic plants. In combinations

Table 3 - Estimates of the variety "per se" (v_i and v_j), of varietal heterosis (h_i and h_j), of general (g_i and g_j) and specific (s_{ij}) combination abilities for percentage of plants asymptomatic to PepYMV, *Phytophthora capsici*, reproduction index (RI), and reproduction factor (RF) of *Meloidogyne incognita* in bell pepper hybrids

μ	PepYMV			Phytophthora capsici			RI			RF		
	72.62 ± 1.40			55.70 ± 1.68			168.01 ± 8.44			34.01 ± 2.03		
d	22.12 ± 1.40			41.58 ± 1.68			96.42 ± 8.44			18.98 ± 2.03		
hm	20.82 ± 1.68			28.94 ± 2.02			-112.58 ± 10.15			-19.50 ± 2.44		
Line of group I	vi	hi	gi = 1/2vi+ hi	vi	hi	gi = 1/2vi + hi	vi	hi	gi = 1/2vi + hi	vi	hi	gi = 1/2vi + hi
1-	-15.92	2.5	-5.46	1.21	1.26	1.87	54.64	-32.61	-5.29	14.44	-9.31	-2.09
2-	5.74	4.17	7.04	1.21	-9.66	-9.06	-66.29	22.59	-10.56	-15.34	4.2	-3.47
3-	5.74	3.62	6.49	-4.84	-5.59	-8.01	19.35	8.31	17.99	6.22	1.7	4.81
4-	5.74	-7.72	-4.85	1.21	10.64	11.25	-90.73	37.81	-7.56	-11	3.84	-1.66
5-	-1.29	-2.58	-3.22	1.21	3.35	3.95	83.02	-36.1	5.42	5.67	-0.43	2.41
Standard error	3.74	2.64		4.48	3.16		22.52	15.92		5.42	3.83	
Line of group II	vj	hj	gj = 1/2vj + hj	vj	hj	gj = 1/2vj + hj	vj	hj	gj = 1/2vj + hj	vj	hj	gj = 1/2vj + hj
1'-	-50	20.75	-4.25	11.46	-4.38	1.35	-70.82	-16.91	-52.34	-14.49	-6.13	-13.37
2'-	-50	15.15	-9.85	19.75	-10.6	-0.73	-68.36	-19.28	-53.48	-14.2	-6.53	-13.65
3'-	50	-17.95	7.05	-15.61	-0.01	-7.81	-70.04	-16.36	-51.38	-14.33	-5.9	-13.05
4'-	50	-17.95	7.05	-15.61	14.99	7.19	209.28	52.56	157.2	43	18.57	40.07
Standard error	3.62	2.43		4.34	2.91		21.8	14.63		5.25	3.52	
						Sij						
1 x 1'	4.83			4.37			4.78			1.96		
1 x 2'	-15.74			6.45			5.37			2.2		
1 x 3'	5.45			-11.5			5.87			2.18		
1 x 4'	5.45			0.63			-16.03			-6.34		
2 x 1'	4.25			6.97			8.1			2.86		
2 x 2'	9.84			-11.8			12.38			3.79		
2 x 3'	-7.04			-4.68			7.9			2.72		
2 x 4'	-7.04			9.47			-28.38			-9.39		
3 x 1'	4.8			1.77			-18.66			-5.02		
3 x 2'	8.18			-0.31			-18.61			-4.96		
3 x 3'	-6.49			-3.64			-15.71			-3.98		
3 x 4'	-6.49			2.18			52.98			13.97		
4 x 1'	-8.97			1.25			11.15			2.62		
4 x 2'	-0.72			1.25			6.78			1.5		
4 x 3'	4.85			6.25			6.91			1.42		
4 x 4'	4.85			-8.75			-24.84			-5.56		
5 x 1'	-4.91			-14.37			-5.38			-2.43		
5 x 2'	-1.56			4.37			-5.92			-2.54		
5 x 3'	3.23			13.5			-4.97			-2.35		
5 x 4'	3.23			-3.54			16.28			7.32		
Standard error	3.24			3.88			19.5			4.69		

1 = PIX-044B-01-01, 2 = PIX-044B-13-01, 3 = PIX-045B-27-02, 4 = PIX-045B-32-03, 5 = PIX-052B-06-01. 1' = Carolina Wonder, 2' = Charleston Belle, 3' = MYR-29-09-05, 4' = MYR-29-11-08

Table 4 - Estimates of values and relative percentage of heterosis in relation to the average of the parents (HRAP) for percentage of plants asymptomatic to PepYMV, *Phytophthora capsici*, reproduction index (RI), and reproduction factor (RF) of *Meloidogyne incognita* in bell pepper hybrids

Identification of the treatments	HRAP%							
	PepYMV		P.capsici		M. incognita			
	(1)		(2)		RI (3)		RF (4)	
	$M_{F1} - M_p$	%	$M_{F1} - M_p$	%	$M_{F1} - M_p$	%	$M_{F1} - M_p$	%
1 x 1’	48.91	123.31	30.21	47.17	-157.32	-98.38	-32.99	-97.06
1 x 2’	22.73	57.32	26.06	38.22	-159.1	-98.73	-33.15	-97.16
1 x 3’	10.83	12.08	18.75	37.13	-155.69	-97.12	-32.55	-95.54
1 x 4’	10.83	12.08	45.83	90.76	-108.67	-36.22	-16.59	-26.43
2 x 1’	50.00	99.01	21.87	34.16	-98.8	-99.37	-18.56	-97.21
2 x 2’	50.00	99.01	-3.1	-4.55	-96.9	-96.24	-18.03	-93.82
2 x 3’	0.00	0.00	14.58	28.88	-98.46	-98.62	-18.48	-96.38
2 x 4’	0.00	0.00	43.75	86.63	-65.81	-27.48	-6.11	-12.76
3 x 1’	50.00	99.01	20.74	33.99	-139.85	-98.31	-28.96	-96.90
3 x 2’	47.77	94.6	12.42	19.07	-142.87	-99.06	-29.29	-97.60
3 x 3’	0.00	0.00	19.69	41.5	-136.35	-95.57	-27.68	-92.40
3 x 4’	0.00	0.00	40.53	85.38	1.27	0.45	14.75	25.14
4 x 1’	24.87	49.25	36.46	56.93	-80.52	-92.33	-19.17	-90.14
4 x 2’	27.52	54.50	30.23	44.33	-87.27	-98.64	-20.69	-96.70
4 x 3’	0.00	0.00	45.83	90.76	-84.22	-96.12	-20.14	-94.38
4 x 4’	0.00	0.00	45.83	90.76	-47.07	-20.70	-2.65	-5.29
5 x 1’	34.07	72.52	13.54	21.14	-170.99	-98.22	-28.50	-96.28
5 x 2’	31.82	67.74	26.06	38.22	-173.91	-99.18	-29.01	-97.58
5 x 3’	3.52	3.63	45.83	90.76	-170.03	-97.44	-28.20	-94.99
5 x 4’	3.52	3.63	43.75	86.64	-79.84	-25.41	5.96	10.20
DMS	23.27		27.85		139.90		33.78	

1 = PIX-044B-01-01, 2 = PIX-044B-13-01, 3 = PIX-045B-27-02, 4 = PIX-045B-32-03, 5 = PIX-052B-06-01,1’ = Carolina Wonder, 2’ = Charleston Belle, 3’ = MYR-29-09-05, 4’ = MYR-29-11-08. Mp = Mean of the parents; M_{F1} = Mean of the hybrid; HRAP% = heterosis relative to the average of the parents, expressed in %. (1) % of plants asymptomatic after inoculation with PepYMV; (2) % plants asymptomatic after inoculation with *P. capsici;* (3) nematode reproduction index; (4) nematode reproduction factor

of lines of group I with lines 1’ and 2’ of group II (intermediate levels of resistance), the values of HRAP% ranged from +21.14% to +56.93% for crossing with 1’ and from -4.55% to +44.33% for crossing with 2’. Since the allele(s) that control resistance to *P. capsici* in group I have dominant action, a negative value of HRAP% in combinations with 2’ may be indicative of recessive gene action of the alleles that control intermediate resistance to *P. capsici* in 1’ or 2’. The data obtained, however, do not definitively clarify this supposition since the genotypes of the lines are not totally fixed for the trait of resistance to *P. capsici*.

M. incognita

For both the characteristics that evaluated resistance to *M. incognita* (reproduction index - RI, and reproduction factor - RF), differences between groups, among lines within each group (v_i, v_j), and heterosis (Table 2) were significant. Of the components of heterosis, in both cases, average heterosis ($\overline{(i)}$), the varietal heteroses of group II (hj), and, in the case of RI, the varietal heterosis of group I (hi) were significant. The effects of specific heterosis were not significant in either of the two cases. They indicate that the additive effects is more important than the non-additive

effects in expression of resistance to M. incognita, such that the response of the hybrids in regard to M. incognita can be predicted based on the reactions of their parents.

All the lines of group I were confirmed as susceptible to nematodes (Table 5) and the significant differences in the components v_i and h_i can thus be attributed to small differences in the genotypic background of these susceptible lines. However, three of the lines of group II (1', 2', 3') were considered resistant and only one (4'), susceptible (Table 5), a fact that was reflected in the high mean squares for the differences in varietal heterosis within group II (v_j) (Table 2).

The hybrids studied were therefore of two types: those in which one of the parents (1', 2', 3') was resistant, and those in which both parents were susceptible. The hybrids with one resistant parent exhibited all the estimates of HRAP% slightly lower than 100% (Table 4), an indication that the average degree of dominance of the allele(s) that control resistance to *M. incognita* is only slightly lower than 1. The hybrids with both parents susceptible, for their part, were also all susceptible, and the values of HRAP% varied, both for RI and for RF, from values near zero to 37% (Table 4), reflecting some background differences among the susceptible genotypes.

It is known that lines 1' and 2' have the N gene, which confers resistance to *M. incognita* (FERY; DUKES; THIES, 1998). However, the resistant line 3' and the susceptible line 4' were obtained through selection from an open pollination population (MYR-29), which was, a priori, taken as susceptible to *M. incognita*. It is possible that MYR-29 population included a percentage of resistant and susceptible plants to *M. incognita* (Table 5). The gene(s) that confer(s) resistance in 3' (MYR-29-09-05) must be studied to identify whether it or they are alleles of N.

Except for the lines 1=PIX-044B-01-01 (in regard to resistance to PepYMV), 1'=Carolina Wonder and 2'=Charleston Belle (for resistance to *P. capsici*), and 3'=MYR-29-09-05 (in regard to the reproduction index and factor of *M. incognita*), all the others exhibited the presumed reactions in relation to PepYMV, *P. capsici*, and *M. incognita* (Table 5). The hybrids obtained from the combinations between the lines of group I (1, 2, 3, 4, 5) with the lines of group II (1', 2', 3') had multiple resistance to the three phytopathogens considered.

Table 5 - Percentage (%) of plants asymptomatic to PepYMV and to *P. capsici*, reproduction index, and factor of *M. incognita* in bell pepper

Parental lines	PepYMV	P. capsici	M. incognita	
	%[1]	%[1]	RI[1]	RF[1]
		Group I		
1= PIX-044B-01-01	78.3 bcd	100.0 a	318.5 de	66.9 cd
2= PIX-044B-13-01	100.0 a	100.0 a	197.6 bcd	37.1 abcd
3= PIX-045B-27-02	100.0 a	93.9 ab	283.3 cde	58.7 cd
4= PIX-045B-32-03	100.0 a	100.0 a	173.2 bc	41.5 cd
5= PIX-052B-06-01	92.9 abc	100.0 a	346.9 e	58.2 cd
		Group II		
1'= CarolinaWonder	0.0 e	27.1 ef	0.2 a	0.0 a
2'= Charleston Belle	0.0 e	35.4 e	2.7 a	0.3 a
3'= MYR-29-09-05	100.0 a	0.0 g	1.0 a	0.2 a
4'= MYR-29-11-08	100.0 a	0.0 g	280.3 cde	57.6 cd
		Additional		
PIM-013	0.0 e	100.0 a	159.0 bc	34.7 abcd
		Controls		
Criollo de Morellos	100.0 a	100.0 a	1.6 a	0.2 a
Magnata Super	0.0 e	2.1 fg	157.0 bc	37.1 abcd

[1] Mean values followed by the same letter in the column do not differ among themselves by the Tukey test at 5% probability

CONCLUSIONS

1. Additive gene effects were important for all the traits evaluated, however non-additive effects were also important for percentage of plants resistant to PepYMV and *Phytophthora capsici*;
2. The values of heterosis relative to the average of the parents (HRAP%) for resistance to PepYMV and *P. capsici* for most hybrids were in the direction of conferring greater resistance;
3. For the reproduction index (RI) and reproduction factor (RF) of *Meloidogyne incognita*, the values of heterosis relative to the average of the parents (HRAP%) were mostly negative, that is, in the direction of a greater degree of resistance;
4. The alleles that control resistance to PepYMV, to *P. capsici*, and to *M. incognita* have a degree of dominance near 1, in absolute value, which indicates a favorable situation for obtaining hybrids that accumulate multiple resistance to these pathogens.

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