




## Rust effect estimation in soybean crosses for tolerance to Asian rust

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**ABSTRACT:** Asian soybean rust is an important disease that has deeply troubled farmers and researchers since it was first reported. The causal agent, biotrophic fungus *Phakopsora pachyrhizi* Sydow & P. Sydow, has found extremely favorable conditions for its dissemination in Brazil. The most characteristic symptom of infection appears in the leaves, initially on the adaxial leaf surface, as small angular points less than 1mm in diameter, together with gray-colored uredospores (spores). Management involves a set of practices that guarantee coexistence between the plant and the pathogen without significant damage to the crop. The objective of this research was to evaluate tolerance to Asian rust by estimating losses caused by natural infection in the field. Experiments with generation  $F_4$  (2014/15) plants were established in a randomized blocks design with four replicates, with two types of genotypes (crosses and parents) and two schemes for disease management using fungicides. For analysis of the data, a test was applied on two dependent samples to verify the significance of the estimate of the rust effect. With regard to grain yield and tolerance, the most outstanding crosses were 104 (USP14-01-20 × EMGOPA313) and 149 (USP93-05.552 × EMGOPA313). Crosses 147 (USP93-05552 × PI153.282) and 137 (USP70.108 × PI153.282) were graded as tolerant in the evaluation of both yield reduction and seed size. We found evidence of tolerance to Asian rust in both crosses and parents. The statistical test revealed the significance of the rust effect estimates in soybean.

**Key words:** *Glycine max*, *Phakopsora pachyrhizi*, partial diallel, paired t-test, rust managements with fungicides.

### Estimativa do efeito ferrugem em cruzamentos de soja para tolerância à ferrugem asiática

**RESUMO:** A ferrugem asiática da soja é a doença que mais tem preocupado os produtores e pesquisadores desde sua primeira evidência. Ela é causada pelo fungo biotrófico *Phakopsora pachyrhizi* Sydow & P. Sydow que encontrou condições extremamente favoráveis para a sua disseminação no Brasil. O sintoma mais característico apresenta-se nas folhas, inicialmente na face adaxial, como pequenos pontos angulares com menos de um milímetro de diâmetro, juntamente com os uredósporos (esporos) de coloração acinzentada. O manejo se dá por meio de um conjunto de práticas que garanta a boa convivência entre a planta e o patógeno e sem que ocorra danos significativos à lavoura. O trabalho objetivou avaliar a tolerância à ferrugem asiática estimando as perdas causadas pela infestação natural a campo. Os experimentos com a geração  $F_4$  (2014/15) foram delineados em blocos ao acaso com quatro repetições. Foram realizadas categorias de experimentos, envolvendo dois tipos de genótipos (cruzamentos e genitores) e dois tipos de manejos de doenças com fungicidas. Para a análise dos dados foi aplicado um teste, para duas amostras dependentes, a fim de verificar a significância da estimativa do efeito ferrugem. Considerando a produtividade e a tolerância, os cruzamentos mais importantes foram 104 (USP 14-01-20 x EMGOPA 313) e 149 (USP 93-05.552 x EMGOPA 313). Os cruzamentos 147 (USP 93-05.552 x PI 153.282) e 137 (USP 70.108 x PI 153.282) foram tolerantes tanto na avaliação de redução da produtividade e do tamanho de semente. Houveram evidências de tolerância à ferrugem asiática nos cruzamentos e nos genitores. O teste estatístico revelou a significância das estimativas de efeito ferrugem em soja.

**Palavras-chave:** *Glycine max*, *Phakopsora pachyrhizi*, diallelo parcial, teste t pareado, manejo da ferrugem com fungicidas.

### INTRODUCTION

Genetic resistance of soybean cultivars to Asian rust is a key component of integrated management for disease control (HARTMAN et al., 2005; YORINORI et al., 2009). Genetic resistance is a defense response mechanism that counters the

aggressiveness of the causal agent. Exploration of vertical resistance has led to the identification of some of the major genes that control resistance to Asian rust (CALVO et al., 2008; MONTEROS et al., 2007; LI et al., 2012). However, these genes are no longer effective against the fungus strains present in Brazilian crops, since the fungus expresses high

variability and climatic conditions in the country are highly favorable for its development.

An alternative way to minimize the impact of soybean rust on crop productivity is to develop tolerant lines. Tolerance is the ability of the plant to outlive the attack of the pathogen without significant damage to economic yield despite visible symptoms of the disease. Thus, breeding programs aimed to obtain tolerant genotypes have sought to develop effective strategies to assess field tolerance (HARTMAN et al., 2005). One of these involves management schemes based on the application of different types of fungicides to compare genotypes in both the presence and absence of rust; thus, allowing estimation of losses caused by the disease (SCHAFFER, 1971; LEVY, 2004).

This work aimed to evaluate the tolerance of soybean genotypes to Asian rust through the estimation of yield losses and reduction of seed size (SS) caused by natural field infection.

## MATERIALS AND METHODS

The genetic material was derived from a partial diallel, with 50 crosses between two sets of genotypes: group I (10 parents represented by experimental lines with high grain yield and resistance to the most important diseases) and group II (5 parents including lines, cultivars, and a Plant Introduction), details are given in table 1. All parent materials were seeded in a greenhouse to conduct the crosses during the months of January and February

Table 1 - Partial diallel crossings (10 × 5) between two sets of parental genotypes with 10 and 5 members in parental Groups I, and II, respectively. Each parent with its corresponding genealogy and the 50 crosses with identification numbers ranging from 101 to 150 are listed.

Grup I x Grup II <sub>ID</sub> *	(G11) MSOY6101	(G12) PI153.282	(G13) A4725RG	(G14) EMGOPA313	(G15) Caiapônia
				[IAC 7 x (Santa Rosa x GO79-3068)]**	[Primavera (OCEPAR 3) x BR 85-6356]**
(G1) USP 14-01-20 [Cristalina x IAC-4]**	G1xG11 <sub>101</sub>	G1xG12 <sub>102</sub>	G1xG13 <sub>103</sub>	G1xG14 <sub>104</sub>	G1xG15 <sub>105</sub>
(G2) USP 70.004 [(Soc 81-76 x Foster) x (IAC Foscarin 31 x Forrest)]**	G2xG11 <sub>106</sub>	G2xG12 <sub>107</sub>	G2xG13 <sub>108</sub>	G2xG14 <sub>109</sub>	G2xG15 <sub>110</sub>
(G3) USP 70.006 [Foster x FT 79- 3408]**	G3xG11 <sub>111</sub>	G3xG12 <sub>112</sub>	G3xG13 <sub>113</sub>	G3xG14 <sub>114</sub>	G3xG15 <sub>115</sub>
(G4) USP 70.010 [(IAC Foscarin 31 x Forrest) x (Foster x FT 79- 3408)]**	G4xG11 <sub>116</sub>	G4xG12 <sub>117</sub>	G4xG13 <sub>118</sub>	G4xG14 <sub>119</sub>	G4xG15 <sub>120</sub>
(G5) USP 70.042 [(Soc.81-76 x Foster) x Hartwig]**	G5xG11 <sub>121</sub>	G5xG12 <sub>122</sub>	G5xG13 <sub>123</sub>	G5xG14 <sub>124</sub>	G5xG15 <sub>125</sub>
(G6) USP 70.057 [Kirby x FT-2]**	G6xG11 <sub>126</sub>	G6xG12 <sub>127</sub>	G6xG13 <sub>128</sub>	G6xG14 <sub>129</sub>	G6xG15 <sub>130</sub>
(G7) USP 70.080 [(Coker x Primavera) x (Viçosa x IAC-10)]**	G7xG11 <sub>131</sub>	G7xG12 <sub>132</sub>	G7xG13 <sub>133</sub>	G7xG14 <sub>134</sub>	G7xG15 <sub>135</sub>
(G8) USP 70.108 [Hartwig x PI 371.611]**	G8xG11 <sub>136</sub>	G8xG12 <sub>137</sub>	G8xG13 <sub>138</sub>	G8xG14 <sub>139</sub>	G8xG15 <sub>140</sub>
(G9) USP 70.109 [(IAC-6 x UFV-4) x Hartwig]**	G9xG11 <sub>141</sub>	G9xG12 <sub>142</sub>	G9xG13 <sub>143</sub>	G9xG14 <sub>144</sub>	G9xG15 <sub>145</sub>
(G10) USP 93-05.552 [GO 81-8.491 x BR 80-15.725-B]**	G10xG11 <sub>146</sub>	G10xG12 <sub>147</sub>	G10xG13 <sub>148</sub>	G10xG14 <sub>149</sub>	G10xG15 <sub>150</sub>

\*ID: Identification numbers. \*\*Genealogy.

2011. Generations  $F_1$  and  $F_2$  were cultivated in order to multiply the seeds obtained from each crossing.

#### Experimental design

Experiments were carried out involving two types of genotypes (crosses and parents); and two types of disease management schemes based on use of fungicides:

- O & P management, consisting of two successive applications of fungicides that control rust and other fungal diseases, especially end-of-cycle diseases (ECD); Piraclostrobin 133g l<sup>-1</sup> and Epoxiconazole 50g l<sup>-1</sup>, (BASF) and a third application using fungicide Priori Xtra® (Azoxystrobin 200g l<sup>-1</sup> and Ciproconazole 80g l<sup>-1</sup>, Syngenta).

- D management: consisting of applications of a fungicide that controls ECD, except for rust; three applications of fungicide Derosal® 500SC (Carbendazim, 500g l<sup>-1</sup>, Bayer) were used to control ECD.

In summary, in each fungicide management scheme, 65 genotypes (15 parents and 50 crosses) were distributed in four randomized blocks. Each block consisted of one plot. Seeds were sown in rows 3.0m long (1.0m intended for path and 2.0m for sowing) with spacing of 0.5m between rows, for a useful total area of 1.5m<sup>2</sup> per plot.

#### Evaluated characters

The following characters were evaluated:

- Grain yield (GY). Plants in each plot were harvested together. The seeds obtained were pre-dried in a laboratory environment until moisture stabilized at 13%; next, seeds were weighed, data were recorded in g.plot<sup>-1</sup> and later transformed into kg ha<sup>-1</sup>.

- Seed Size (SS). As 100-seed weight is highly and positively correlated with SS and much easier to measure, 100-seed weight was obtained by random sampling of 100 seeds from the material harvested in each plot.

#### Significance of rust effect

Rust effect (RE) for each genotype (parents and crosses) was estimated as the difference between adjusted means of each genotype, between the two experiments involving the two disease management schemes evaluated. RE was estimated for grain yield (REGY) and for 100-seed weight (RESS), using the following formulae: REGY = GY (D) - GY (O & P) and RESS = SS (D) - SS (O & P).

Rust reaction rate RRR (%) was estimated for GY and for SS by the following formula:

$$\left| \frac{\bar{D} - \bar{O\&P}}{\bar{D}} * 100 \right|$$

In order to evaluate the significance of RE, the dependent (paired) samples test was used (STEEL & TORRIE, 1980). This test is commonly used when the same group of elements is subjected to some treatment under two different situations; i.e., two different disease management schemes, in our case. The hypotheses tested were:  $H_0: \mu_1 - \mu_2 = 0$ ;  $H_1: \mu_1 - \mu_2 \neq 0$ ; where,  $\mu_1$  is the average character in O & P management; and is the mean of the character in the D management. The test statistic is given by:

$$\frac{\bar{d} - \mu_0}{\frac{S_d}{\sqrt{n}}}$$

where,  $\bar{d}$  is the mean of the differences between the two managements, given by:

$$\bar{d} = \bar{Y}_1 - \bar{Y}_2 = \frac{1}{n} \sum_{i=1}^n d_i = \frac{1}{n} \sum_{i=1}^n (Y_{i1} - Y_{i2}), \quad i = 1, 2, \dots, n$$

where:  $S_d$  is the standard deviation of the differences between the two managements, given by:

$$S_d^2 = \frac{1}{n-1} \left[ \sum_{i=1}^n d_i^2 - \frac{(\sum_{i=1}^n d_i)^2}{n} \right]$$

It is worth noting that the test statistic for the test for equality of two independent means is variance, which is calculated considering all the differences between the dependent observations. Value of the minimum significant difference for GY and SS was consistently estimated at 5%, considering the Bonferroni adjustment, in order to obtain the significance of the RE for each cross.

Analysis of variance involving the source of variation (fungicide) was performed for the randomized blocks design according to the model below:

$$Y_{ijl} = \mu + g_i + b_j(f)_l + f_l + (gf)_{il} + e_{ijl}$$

Where,  $Y_{ijl}$  is the observed value of each genotype in the block and in the fungicide.  $\mu$  is the fixed effect of the general mean of the experiment.  $g_i$  is the fixed effect of the genotype, being ( $i = 1, \dots, g$ ).  $b_j(f)_l$  is the random effect of the block within the fungicide.  $f_l$  is the fixed effect of the fungicide, where ( $l = 1, \dots, f$ ).  $(gf)_{il}$  is the fixed effect of the interaction between genotype and fungicide and  $e_{ijl}$  is the random effect of the experimental residue of the plot that received the genotype within the block in the fungicide, assuming that residues are independent and normally distributed with mean zero and variance  $\sigma^2$ . Finally, we used the method by Scott & Knott (1974) in the analysis for grouping of means.

## RESULTS AND DISCUSSION

Although, environmental conditions proved favorable for the development of the fungus, disease symptoms were observed rather

late in the growing season. Before the analysis of variance for grain yield (GY) and 100-seed weight (SS) (Table 2), means among disease management were different from each other. However, considering the interactions Genotype  $\times$  Fungicide (G  $\times$  F), these were important only for GY, since there was a strong influence of the fungicide on the mean values of GY. The average loss of GY in the presence of rust was 358kg ha<sup>-1</sup>.

In principle, tolerance entails null or negligible GY loss in the presence of the rust and in the absence of any fungicide application (KAWUKI et al., 2004). Therefore, null or negative rust reaction rate (RRR) values indicate sensitivity to the fungus, while any variation in the negative RRR values, indicated the level of tolerance of each genotype. Conversely, positive and significant values of RRR indicate sensitivity to fungicide (phytotoxicity reaction), while intermediate values of RRR indicate moderate tolerance (ARAÚJO & VELLO, 2009).

Table 3 shows mean GY and rust effect on GY for the 50 crosses under scrutiny. The five crosses with the highest mean grain yield were 146 (USP93-05.552  $\times$  MSOY6101), 150 (USP93-05.552  $\times$  Caiapônia), 130 (USP70.057  $\times$  Caiapônia), 105 (USP14-01-20  $\times$  Caiapônia) and 102 (USP14-01-20  $\times$  PI153.282), whose means were above the overall mean. Among these crosses, only 146, 105, and 102 showed reduction of mean GY attributable to rust incidence. The outstanding performance by cross 146, which in average lost only 267kg ha<sup>-1</sup> in the

presence of Asian rust; thus, maintaining a high mean yield despite the presence of the fungus.

The rust effect on GY ranged from 3.02kg ha<sup>-1</sup> (0.14%) to 1533kg ha<sup>-1</sup> (85.03%), while the mean loss was 426kg ha<sup>-1</sup>. Among the 50 crosses tested, 38 showed significant GY losses. In relation to tolerance, outstanding crosses were: 140 (USP70.108  $\times$  Caiapônia), 138 (USP70.108  $\times$  A4725RG), 133 (USP70.080  $\times$  A4725RG), 147 (USP93-05552  $\times$  PI 153.282), 137 (USP70.108  $\times$  PI153.282), 117 (USP70.010  $\times$  PI153.282), 107 (USP70.004  $\times$  PI153.282), 104 (USP14-01-20  $\times$  EMGOPA313), 149 (USP93-05.552  $\times$  EMGOPA 313), 145 (USP70.109  $\times$  Caiapônia), 129 (USP70.057  $\times$  EMGOPA313) and 141 (USP70.109  $\times$  MSOY6101).

Table 4 shows mean GY and rust effect on GY of the 15 parental genotypes. Parents with characteristics of tall plants showed high productivity, these included: USP14-01-20 (3210kg ha<sup>-1</sup>) and USP93-05.552 (3440kg ha<sup>-1</sup>) from group I; MSOY6101 (2471kg ha<sup>-1</sup>) and Caiapônia (2386kg ha<sup>-1</sup>) from group II.

Genotypes that stood out the most with respect to rust tolerance were parents PI153.282 and A4725RG. However, it is not possible to say that these parents actually expressed tolerance-related genes, since these materials were harvested early and might simply have escaped the period of higher rust incidence. This is why early cultivars have been preferred in the past (HARTMAN et al., 2005). Indeed, KAWUKI et al. (2004) and MELO et al. (2015) evaluated the tolerance of soybean genotypes

Table 2 - Analysis of variance of parents and crosses in the F<sub>4</sub> generation in both fungicide management schemes for grain yield (GY) and seed size (SS). The experiment was laid out in a randomized blocks design with four replicates.

Sources of variation	DF	Mean Squares	
		GY (kg ha <sup>-1</sup> )	SS(g)
Replicates/Fungicides	6	1531329**	6.86***
Fungicides (F)	1	16555463***	44.8***
Genotypes	64	5615858***	13.8***
Crosses (C)	49	5311628***	11.2***
Parents (P)	14	6776801***	24.3***
C vs P	1	4269907*	5.73*
Genotypes x Fungicides	64	685076**	1.36 <sup>ns</sup>
C x Fungicides	49	578464.0	1.27 <sup>ns</sup>
P x Fungicides	14	658348.0	1.62 <sup>ns</sup>
(C vs P) x Fungicides	1	4507023**	2.61 <sup>ns</sup>
Error	381	426400	1.68
CV (%)		31.81	9.44

ns: non-significant; . , \* , \*\* , \*\*\* significant at 10%, 5%, 1% e 0,1% probability, respectively, for the F test.



Table 3 - Grain yield (GY, kg ha<sup>-1</sup>) in the O & P and D disease management schemes, rust effect (RE), rust reaction rate (RRR) and mean GY of 50 crosses from F<sub>4</sub> generation.

Genotypes	ID <sup>a</sup>	GY (O&P)	GY (D)	RE		RRR (%)	Mean GY <sup>**</sup>	
USP 70.108 x Caiapônia	140	2356.67	2360.00	3	ns	0.14	2358.34	b
USP 70.108 x A4725RG	138	2003.33	2010.00	7	ns	0.33	2006.67	b
USP 70.080 x A4725RG	133	2053.33	2040.00	-13	ns	-0.65	2046.67	b
USP 93-05.552 x PI 153.282	147	683.33	663.33	-20	ns	-3.02	673.33	d
USP 70.108 x PI 153.282	137	840.00	863.33	23	ns	2.70	851.67	d
USP 70.010 x PI 153.282	117	576.67	540.00	-37	ns	-6.79	558.34	d
USP 70.004 x PI 153.282	107	373.33	430.00	57	ns	13.18	401.67	d
USP 14-01-20 x EMGOPA 313	104	2786.67	2723.33	-63	ns	-2.33	2755.00	a
USP 93-05.552 x EMGOPA 313	149	2686.67	2753.33	67	ns	2.42	2720.00	a
USP 70.109 x Caiapônia	145	1856.67	1926.67	70	ns	3.63	1891.67	b
USP 70.057 x EMGOPA 313	129	2120.00	2030.00	-90	ns	-4.43	2075.00	b
USP 70.109 x MSOY 6101	141	2350.00	2233.33	-117	ns	-5.22	2291.67	b
USP 70.080 x Caiapônia	135	2840.00	2700.00	-140	*	-5.19	2770.00	a
USP 70.006 x A4725RG	113	1666.67	1810.00	143	*	7.92	1738.34	b
USP 70.006 x PI 153.282	112	603.33	433.33	-170	*	-39.23	518.33	d
USP 70.109 x PI 153.282	142	546.67	716.67	170	*	23.72	631.67	d
USP 70.042 x PI 153.282	122	460.00	283.33	-177	*	-62.35	371.67	d
USP 70.108 x EMGOPA 313	139	2143.33	2320.00	177	*	7.61	2231.67	b
USP 70.010 x A4725RG	118	1393.33	1193.33	200	*	8.30	1293.33	c
USP 70.057 x PI 153.282	127	766.67	566.67	-200	*	-16.76	666.67	d
USP 70.006 x MSOY 6101	111	2210.00	2410.00	-200	*	-35.29	2310.00	b
USP 93-05.552 x A4725RG	148	2316.67	2080.00	-237	*	-11.38	2198.34	b
USP 70.109 x EMGOPA 313	144	1146.67	1400.00	253	*	18.10	1273.34	c
USP 93-05.552 x MSOY 6101	146	3520.00	3253.33	-267	*	-8.20	3386.67	a
USP 70.004 x A4725RG	108	983.33	1263.33	280	*	22.16	1123.33	c
USP 70.057 x MSOY 6101	126	1733.33	2030.00	297	*	14.61	1881.67	b
USP 70.042 x A4725RG	123	1083.33	1393.33	310	*	22.25	1238.33	c
USP 70.080 x MSOY 6101	131	2990.00	2653.33	-337	*	-12.69	2821.67	a
USP 70.004 x Caiapônia	110	2893.33	2536.67	-357	*	-14.06	2715.00	a
USP 70.042 x Caiapônia	125	2210.00	1853.33	-357	*	-19.24	2031.67	b
USP 70.109 x A4725RG	143	1566.67	1183.33	-383	*	-32.39	1375.00	c
USP 70.006 x Caiapônia	115	2496.67	2100.00	-397	*	-18.89	2298.34	b
USP 70.057 x Caiapônia	130	2843.33	3283.33	440	*	13.40	3063.33	a
USP 70.042 x MSOY 6101	121	1556.67	1073.33	-483	*	-45.03	1315.00	c
USP 70.080 x PI 153.282	132	3116.67	2590.00	-527	*	-20.33	2853.34	a
USP 70.080 x EMGOPA 313	134	2753.33	2216.67	-537	*	-24.21	2485.00	b
USP 70.057 x A4725RG	128	1880.00	2456.67	577	*	23.47	2168.34	b
USP 93-05.552 x Caiapônia	150	2776.67	3353.33	577	*	17.20	3065.00	a
USP 70.108 x MSOY 6101	136	1956.67	2570.00	-613	*	-55.76	2263.34	b
USP 14-01-20 x A4725RG	103	3240.00	2533.33	-707	*	-27.89	2886.67	a
USP 70.042 x EMGOPA 313	124	2560.00	1776.67	-783	*	-44.09	2168.34	b
USP 70.010 x MSOY 6101	116	3293.33	2496.67	-797	*	-31.91	2895.00	a
USP 70.004 x MSOY 6101	106	2643.33	1840.00	-803	*	-43.66	2241.67	b
USP 14-01-20 x Caiapônia	105	3416.67	2576.67	-840	*	-32.60	2996.67	a
USP 70.006 x EMGOPA 313	114	2430.00	1386.67	-1043	*	-75.24	1908.34	b
USP 70.004 x EMGOPA 313	109	2510.00	1230.00	-1280	*	-104.07	1870.00	b
USP 70.010 x EMGOPA 313	119	2993.33	1643.33	-1350	*	-82.15	2318.33	b
USP 14-01-20 x PI 153.282	102	3660.00	2298.33	-1362	*	-59.25	2979.17	a
USP 14-01-20 x MSOY 6101	101	3273.33	1820.00	-1453	*	-79.85	2546.67	a
USP 70.010 x Caiapônia	120	3336.67	1803.33	-1533	*	-85.03	2570.00	a

<sup>a</sup>Least significant difference within the same genotype in the two fungicides: 139kg ha<sup>-1</sup> (5%). ID: Identification numbers (To identify genotypes, see Table 1) <sup>\*\*</sup>Averages followed by the same letter are joined in the same group by Scott & Knott test at 5% significance.

Table 4 - Grain yield (GY, kg ha<sup>-1</sup>) in the O & P and D disease management schemes, rust effect (RE), rust reaction rate (RRR) and mean GY of the 15 parental genotypes.

Genotypes	ID <sup>a</sup>	GY (O&P)	GY (D)	RE		RRR (%)	Mean GY <sup>**</sup>	
PI 153.282	G12	55.13	12.13	-43	ns	-354.40	33.63	d
A4725RG	G13	553.33	476.67	-77	ns	-16.08	515.0	d
USP 70.042	G5	1660.00	1823.33	163	*	8.96	1741.6	b
USP 93-05.552	G10	3640.00	3240.00	-400	*	-12.35	3440.0	a
USP 70.010	G4	2768.33	2220.00	-548	*	-24.70	2494.1	b
USP 70.004	G2	1713.33	1100.00	613	*	23.87	1406.6	c
USP 14-01-20	G1	3550.00	2870.00	-680	*	-23.69	3210.0	a
MSOY 6101	G11	2846.67	2096.67	-750	*	-35.77	2471.6	b
USP 70.109	G9	2576.67	1706.67	-870	*	-50.98	2141.6	b
EMGOPA 313	G14	2950.00	2063.33	-887	*	-42.97	2506.6	b
CAIAPÔNIA	G15	2833.33	1940.00	-893	*	-46.05	2386.6	b
USP 70.006	G3	2263.33	1273.33	-990	*	-77.75	1768.3	b
USP 70.057	G6	3540.00	2283.33	-1257	*	-55.04	2911.6	a
USP 70.080	G7	3483.33	2046.67	-1437	*	-70.20	2765.0	a
USP 70.108	G8	4053.33	1886.67	-2167	*	-114.84	2970.0	a

<sup>a</sup>Least significant difference within the same genotype in the two fungicides: 139kg ha<sup>-1</sup> (5%). ID: Identification numbers (To identify genotypes, see Table 1). <sup>\*\*</sup>Averages followed by the same letter are joined in the same group by Scott & Knott test at 5% significance.

to rust and found that early genotypes were more tolerant than intermediate and late genotypes.

Other studies have reported (TICHAGWA, 2004; YANG et al., 1992) that, besides reduced GY, rust attack causes reduced SS. Therefore, as it reflects SS, the mean 100-seed weight and the rust effect on mean 100-seed weight were calculated for all crosses (Table 5). Five crosses showed the highest SS: 130 (USP70.057 × Caiapônia), 128 (USP70.057 × A4725RG), 110 (USP70.004 × Caiapônia), 122 (USP70.042 × PI153.282) and 140 (USP70.108 × Caiapônia). Crossing 130 (15.95g) reflected SS of USP70.057 (16.87g) and Caiapônia (14.61g) parents and had excellent productivity. However, SS was reduced by almost 1.0g in the presence of rust, unlike that of the cross 110, whose SS was hardly affected, maintaining a mean SS of 15.3g for 100-seed weight.

Nevertheless, large seeds are not necessarily any guarantee of an increase in grain yield, nor are they preferred by most farmers (GIRARD, 2002). Preference for small SS continues among producers, due to the economy with regard to inoculation, treatment, transport, and seed acquisition (THOMAS & COSTA, 2010).

The rust effect on SS ranged from 0.06g (0.48%) to 2.74g (20.4%), while the mean loss was 0.78g. In our experiments, the most tolerant crosses were 142 (USP70.109 × PI153.282), 143 (USP70.109 × A4725RG), 127 (USP70.057 × PI153.282), 136

(USP70.108 × MSOY6101), 132 (USP70.080 × PI153.282), 111 (USP70.006 × MSOY6101), 135 (USP70.080 × Caiapônia), 134 (USP70.080 × EMGOPA313), 147 (USP93-05.552 × PI153.282), 137 (USP70.108 × PI153.282), 110 (USP70.004 × Caiapônia) and 131 (USP70.080 × MSOY6101).

Among parental materials included in the present study, none showed non-significant reduction in SS (Table 6). However, among parents with larger SS, Caiapônia also showed the lowest reduction in SS when compared to the rest of parents.

## CONCLUSION

The statistical test allowed us to establish on a sound basis the significance of the estimates of rust effect in soybean; thus, providing guarantee of the reliability of the information related to the tolerance of the genotypes under study. The most tolerant crosses were: 133 (USP70.080 × A4725RG), 138 (USP70.108 × A4725RG), 140 (USP70.108 × Caiapônia) and 147 (USP93-05.552 × PI153.282). Cross 140 deserve special mention, since its yield surpassed overall mean yield of all crosses. The USP93-05.552 was the most outstanding parental material among the most productive parents identified by the clustering test for high grain yield and low yield loss.

As for SS, crosses with large seed and rust tolerance were 136 (USP70.108 × MSOY6101),

Table 5 - Seed size (SS, g of 100-seed weight) in the O & P and D disease management schemes, rust effect (RE), rust reaction rate (RRR) and mean of SS of the 50 crosses from F<sub>4</sub> generation.

Genotypes	ID <sup>a</sup>	SS (O&P)	SS (D)	RE		RRR(%)	Mean SS <sup>**</sup>	
USP 70.109 x PI 153.282	142	13.74	13.67	-0.06	ns	-0.48	13.71	b
USP 70.109 x A4725RG	143	12.86	12.79	-0.07	ns	-0.57	12.83	c
USP 70.057 x PI 153.282	127	13.78	13.86	0.07	ns	0.54	13.82	b
USP 70.108 x MSOY 6101	136	14.68	14.78	0.11	ns	0.71	14.73	a
USP 70.080 x PI 153.282	132	13.29	13.17	-0.13	ns	-0.95	13.23	c
USP 70.006 x MSOY 6101	111	14.89	14.76	-0.13	ns	-0.86	14.83	a
USP 70.080 x Caiapônia	135	13.37	13.24	-0.13	ns	-1.00	13.31	c
USP 70.080 x EMGOPA 313	134	10.93	10.80	-0.14	ns	-1.27	10.87	d
USP 93-05.552 x PI 153.282	147	13.37	13.54	0.17	ns	1.26	13.46	b
USP 70.108 x PI 153.282	137	14.60	14.77	0.17	ns	1.17	14.69	a
USP 70.004 x Caiapônia	110	15.39	15.22	-0.18	ns	-1.17	15.31	a
USP 70.080 x MSOY 6101	131	11.38	11.56	0.18	ns	1.54	11.47	d
USP 70.006 x EMGOPA 313	114	12.04	11.82	-0.22	*	-1.88	11.93	d
USP 70.109 x MSOY 6101	141	15.02	15.25	0.23	*	1.49	15.14	a
USP 93-05.552 x Caiapônia	150	14.55	14.29	-0.26	*	-1.84	14.42	b
USP 70.109 x Caiapônia	145	14.12	13.85	-0.26	*	-1.89	13.99	b
USP 70.042 x EMGOPA 313	124	13.75	13.48	-0.26	*	-1.97	13.62	b
USP 70.006 x Caiapônia	115	14.91	14.61	-0.30	*	-2.07	14.76	a
USP 70.006 x PI 153.282	112	14.42	14.10	-0.32	*	-2.29	14.26	b
USP 70.109 x EMGOPA 313	144	12.18	12.63	0.45	*	3.56	12.41	c
USP 70.004 x PI 153.282	107	14.83	14.37	-0.46	*	-3.20	14.60	a
USP 70.108 x Caiapônia	140	15.40	14.93	-0.47	*	-3.11	15.17	a
USP 70.042 x Caiapônia	125	14.48	14.00	-0.48	*	-3.41	14.24	b
USP 70.108 x A4725RG	138	14.66	15.17	0.50	*	3.31	14.92	a
USP 14-01-20 x Caiapônia	105	14.67	14.15	-0.52	*	-3.68	14.41	b
USP 70.004 x EMGOPA 313	109	13.88	13.36	-0.52	*	-3.89	13.62	b
USP 70.057 x A4725RG	128	15.76	15.23	-0.52	*	-3.45	15.49	a
USP 93-05.552 x EMGOPA 313	149	11.38	11.90	0.53	*	4.41	11.64	d
USP 70.004 x MSOY 6101	106	15.15	14.62	-0.53	*	-3.63	14.89	a
USP 14-01-20 x EMGOPA 313	104	13.38	12.83	-0.54	*	-4.25	13.11	c
USP 70.004 x A4725RG	108	15.06	14.50	-0.56	*	-3.90	14.78	a
USP 70.057 x MSOY 6101	126	12.92	13.53	0.61	*	4.51	13.23	c
USP 70.108 x EMGOPA 313	139	11.05	11.80	0.75	*	6.33	11.43	d
USP 70.010 x EMGOPA 313	119	12.09	11.29	-0.80	*	-7.09	11.69	d
USP 70.042 x A4725RG	123	14.22	13.40	-0.82	*	-6.14	13.81	b
USP 70.057 x EMGOPA 313	129	11.46	12.30	0.83	*	6.79	11.88	d
USP 70.057 x Caiapônia	130	16.42	15.50	-0.92	*	-5.95	15.96	a
USP 70.080 x A4725RG	133	13.46	12.46	-1.00	*	-7.99	12.96	c
USP 14-01-20 x MSOY 6101	101	13.89	12.84	-1.05	*	-8.18	13.37	c
USP 70.010 x Caiapônia	120	14.87	13.77	-1.10	*	-7.99	14.32	b
USP 14-01-20 x PI 153.282	102	14.62	13.48	-1.14	*	-8.42	14.05	b
USP 93-05.552 x MSOY 6101	146	13.85	12.63	-1.22	*	-9.62	13.24	c
USP 70.010 x MSOY 6101	116	15.48	14.20	-1.28	*	-9.00	14.84	a
USP 70.006 x A4725RG	113	14.92	13.56	-1.36	*	-10.05	14.24	b
USP 93-05.552 x A4725RG	148	14.65	13.26	-1.39	*	-10.47	13.96	b
USP 70.042 x PI 153.282	122	15.90	14.43	-1.47	*	-10.15	15.17	a
USP 70.010 x A4725RG	118	15.09	13.16	-1.92	*	-14.61	14.13	b
USP 70.010 x PI 153.282	117	14.98	12.60	-2.38	*	-18.87	13.79	b
USP 70.042 x MSOY 6101	121	14.39	11.83	-2.56	*	-21.64	13.11	c
USP 14-01-20 x A4725RG	103	16.17	13.43	-2.74	*	-20.41	14.80	a

\*Least significant difference within the same genotype in the two fungicides: 0.19kg ha<sup>-1</sup> (5%). ID: Identification numbers (To identify genotypes, see Table 1). \*\*Averages followed by the same letter are joined in the same group by Scott & Knott test at 5% significance.

111 (USP70.006 × MSOY6101), 137 (USP70.108 × PI153.282) and 110 (USP70.004 × Caiapônia). Crosses with smaller seed and rust tolerance were 134 (USP70.080 × EMGOPA313) and 131 (USP70.080 × MSOY6101).

Crosses 147 (USP93-05552 × PI153.282) and 137 (USP70.108 × PI153.282) showed rust tolerance in the evaluation of both yield reduction and SS. Therefore, they were considered promising for the selection of soybean plants with Asian rust tolerance.

Table 6 - Seed size (SS, g of 100-seed weight) in the O & P and D disease management schemes, rust (EF), rust reaction rate (RRR) and mean of SS of the 15 parents.

Genotypes	ID <sup>a</sup>	SS (O&P)	SS (D)	RE	RRR(%)	Mean SS **		
CAIAPÔNIA	G15	14.82	14.40	-0.42	*	-2.92	14.61	a
USP 70.042	G5	13.53	13.10	-0.43	*	-3.26	13.32	c
USP 14-01-20	G1	13.93	13.43	-0.51	*	-3.76	13.68	b
EMGOPA 313	G14	9.81	9.25	-0.56	*	-6.00	9.53	e
USP 70.057	G6	16.55	17.20	0.65	*	3.79	16.88	a
USP 70.004	G2	14.56	13.84	-0.71	*	-5.17	14.2	b
USP 93-05.552	G10	12.53	13.47	0.94	*	7.00	13	c
A4725RG	G13	15.21	13.86	-1.34	*	-9.68	14.535	a
USP 70.080	G7	12.42	11.06	-1.37	*	-12.34	11.74	d
MSOY 6101	G11	15.43	14.06	-1.37	*	-9.77	14.75	a
USP 70.010	G4	12.56	11.17	-1.40	*	-12.49	11.87	d
USP 70.109	G9	14.72	13.07	-1.65	*	-12.61	13.89	b
USP 70.006	G3	13.66	11.85	-1.82	*	-15.34	12.76	c
USP 70.108	G8	15.74	13.50	-2.23	*	-16.53	14.62	a
PI 153.282	G12							

<sup>a</sup>Least significant difference within the same genotype in the two fungicides: 0.19kg ha<sup>-1</sup> (5%). ID: Identification numbers (To identify genotypes, see Table 1). <sup>\*\*</sup>Averages followed by the same letter are joined in the same group by Scott & Knott test at 5% significance.

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## REFERENCES

ARAÚJO, M.M. de; VELLO, N.A. Characterization of soybean genotypes for Asian soybean rust reaction. **Crop Breeding and Applied Biotechnology**, Londrina, v. 10, n. 3, p. 197–203, 2010. Available from: <[http://www.scielo.br/scielo.php?script=sci\\_artext&pid=S1984-70332010000300003](http://www.scielo.br/scielo.php?script=sci_artext&pid=S1984-70332010000300003)>. Accessed: Jul. 20, 2017. doi: 10.1590/S1984-70332010000300003.

CALVO, E.S. et al. Two major recessive soybean genes conferring soybean rust resistance. **Crop Science**, Madison, v. 48, n. 4, p. 1350-1354, 2008. Available from: <<http://dx.doi.org/10.2135/cropsci2007.10.0589>>. Accessed: Jul. 20, 2017. doi: 10.2135/cropsci2007.10.0589.

GIRARD, R.E. **Estratégias de marketing no agronegócio de semente de soja**. 2002. 124f. Dissertação (Mestrado em Engenharia de Produção) - Universidade Federal de Santa Catarina, Florianópolis. 2002. Available from: <<https://repositorio.ufsc.br/bitstream/handle/123456789/82343/188424.pdf?sequence=1>>. Accessed: Mar. 13, 2017.

HARTMAN, G.L. et al. Breeding for resistance to soybean rust. **Plant Disease**, St. Paul, v. 89, p. 664–666, 2005. Available from: <<https://apsjournals.apsnet.org/doi/pdf/10.1094/PD-89-0664>>. Accessed: Mar. 13, 2017. doi: 10.1094/PD-89-0664

KAWUKI, R.S. et al. Soybean rust severity, rate of rust development and tolerance as influenced by maturity period and season. **Crop Protection**, v.23, p.447-455, 2004. Available from: <<https://www.sciencedirect.com/science/article/pii/S0261219403002412?via%3Dihub>>. Accessed: Apr. 4, 2017. doi: 10.1016/j.cropro.2003.09.016.

LEVY, C. Zimbabwe – a country report on soybean rust control. In: VII WORLD SOYBEAN RESEARCH CONFERENCE e III CONGRESSO BRASILEIRO DE SOJA, 3, 2004, Foz do Iguaçu, PR. **Proceedings...** Londrina: Embrapa, 2004. p.340-348.

LI, S. et al. Identification of a new soybean rust resistance gene in PI567102B. **Theoretical and Applied Genetics**, v. 125, p. 133-142, 2012. Available from: <<https://link.springer.com/article/10.1007/s00122-012-1821-y>>. Accessed: Apr. 4, 2017. doi: 10.1007/s00122-012-1821-y.

MELO, C.L.P. de et al. Tolerance of soybean genotypes to Asian rust. **Ciência Rural** [online], vol.45, n.8, p.1353-1360, 2015. Available from: <<http://dx.doi.org/10.1590/0103-8478cr20141309>>. Accessed: Apr. 12, 2017. doi: 10.1590/0103-8478cr20141309.

MONTEROS, M.J. et al. Mapping and confirmation of the 'Hyyuga' red-brown lesion resistance gene for Asian soybean rust. **Crop Science**, Madison, v. 47, n. 2, p. 829-836, 2007. Available from: <<https://www.scopus.com/record/display.uri?eid=2-s2.0-34247184723&origin=inward&txGid=b2f190feade736da0fbc4e8f35767976>>. Accessed: Mar. 12, 2017. doi: 10.2135/cropsci06.07.0462.

SCHAFER, J.F. Tolerance to plant disease. **Annual Review of Phytopathology**, Palo Alto, v. 9, p.235-252, 1971. Available from: <<http://www.annualreviews.org/doi/abs/10.1146/annurev.py.09.090171.001315>>. Accessed: Mar. 13, 2017. doi: 10.1146/annurev.py.09.090171.001315.



SCOTT, A.J.; KNOTT, M. Cluster analysis method for grouping means in the analysis of variance. **Biometrics**, Arlington, v. 30, n. 3, p. 507–512, 1974. Available from: <<http://www.jstor.org/stable/pdf/2529204.pdf?refreqid=excelsior%3A0ce8a2bdc90db75f1aaaaa8129488166>>. Accessed: Jan. 29, 2018.

STEEL, R.G.D.; TORRIE, J.H. **Principles and Procedures of Statistics**, 2<sup>nd</sup> ed, New York: McGraw-Hill. 1980. 633p.

TICHAGWA, J.S. Breeding for resistance to soybean rust in Zimbabwe. In: WORLD SOYBEAN RESEARCH CONFERENCE, 7., INTERNATIONAL SOYBEAN PROCESSING AND UTILIZATION CONFERENCE, 6.,

CONGRESSO BRASILEIRO DE SOJA, 3., 2004, Foz do Iguaçu, PR. **Proceedings...**, Londrina: Embrapa, 2004. p. 349- 353.

THOMAS, A.L.; COSTA, J.A. **Soja: Manejo para alta produtividade de grãos**. Porto Alegre: Evangraf, 2010. 248p.

YANG, X.B. et al. Comparing the effects of rust on plot yield, plant yield, yield components, and vegetative parts of soybean. **Journal of Phytopathology**, Berlin, v. 136, n. 1, p. 46-56, 1992. Available from: <<http://onlinelibrary.wiley.com/doi/10.1111/j.1439-0434.1992.tb01280.x/pdf>>. Accessed: Jan. 29, 2018. doi: 10.1111/j.1439-0434.1992.tb01280.x.

YORINORI, J.T. et al. Doenças da Soja. **Boletim de Pesquisa da Soja**, Rondonópolis, v. 13, p. 180–228, 2009.