

# Identifying cotton genotypes resistant to *Meloidogyne incognita* race 3 using Blup<sup>1</sup>

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## ABSTRACT

Few genetic resistance sources to root-knot nematodes are known for cotton, and there are no cultivars combining both resistance and good agronomic attributes in Brazil. Techniques that allow an efficient selection of promising sources of genetic resistance are needed. This study aimed to identify cotton genotypes resistant to *Meloidogyne incognita* race 3 via REML/Blup. The experiment was completely randomized, with 32 genotypes and seven replicates, in a protected environment. The statistical model 83 was used. The root weight, final population, reproduction factor and reproduction index were estimated. The final population and reproduction index presented the highest values for genotypic variance and genetic variation coefficients, indicating a favorable situation for the selection of cotton genotypes resistant to the nematode. The mean heritability ( $h^2_{mg}$ ) of the genotype was higher than 85 % for the final population, reproduction factor and reproduction index, opening a possibility of selection success based on genotype means. The genotypes CS8601, SA2572, Coodetec 404 and BJ 3128 are promising for crosses aiming the selection of genotypes resistant to the nematode.

**KEYWORDS:** *Gossypium* spp., genetic resistance, root-knot nematode.

## INTRODUCTION

The nematodes *Meloidogyne incognita* race 3 (Kofoid and White) Chitwood and *Rotylenchus reniformis* represent important phytosanitary challenges for cotton cropping worldwide. However, the root-knot nematode (RKN) is the most important phytoparasite of this crop, because it causes considerable losses in yield and profitability and increases damages caused by other soilborne diseases such as *Fusarium* wilt (Katsantonis et al. 2003, Starr et al. 2007, Mota et al. 2013, Kumar et al. 2016).

## RESUMO

Identificação de genótipos de algodoeiro resistentes a *Meloidogyne incognita* raça 3 usando Blup

Há poucas fontes de resistência genética a nematoides-de-galha relatadas em algodoeiro, e não há cultivares no Brasil que combinem simultaneamente resistência e bons atributos agrônômicos. Técnicas que possibilitem selecionar eficientemente fontes de resistência genética promissoras são necessárias. Objetivou-se identificar genótipos de algodoeiro resistentes a *Meloidogyne incognita* raça 3, por meio de REML/Blup. O experimento foi inteiramente casualizado, com 32 genótipos e sete repetições, em ambiente protegido. Foi utilizado o modelo estatístico 83. Foram estimados a massa de raízes, população final, fator de reprodução e índice de reprodução. A população final e o índice de reprodução apresentaram os maiores valores de variância genotípica e coeficientes de variação genética, sinalizando situação favorável à seleção de genótipos de algodão resistentes ao nematoide. A herdabilidade média do genótipo ( $h^2_{mg}$ ) foi superior a 85 % para população final, fator de reprodução e índice de reprodução, abrindo a possibilidade de sucesso de seleção com base nas médias dos genótipos. Os genótipos CS8601, SA2572, Coodetec 404 e BJ 3128 são promissores para cruzamentos visando à seleção de genótipos resistentes ao nematoide.

**PALAVRAS-CHAVE:** *Gossypium* spp., resistência genética, nematoide-de-galha.

The RKN causes several damages to cotton, either in the root system or in shoots, and may influence physiological processes and plant morphology (Lu et al. 2014, Ma et al. 2014).

The RKN management is mainly performed by crop rotation and chemical nematicides (Van Biljon et al. 2015). Crop rotation is becoming increasingly difficult to carry out, because the RKN is largely distributed in producing areas and counts a great number of hosts. The use of nematicides has been discouraged due to their short-lasting effect and the potential restrictions on the use of chemical pesticides.

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Adopting RKN-resistant cultivars is the most sustainable way to manage this disease, as there are no negative socio-environmental effects, and it is an efficient, durable and easy to implement technology (Starr et al. 2007). Wheeler et al. (2014) reported that partially resistant cultivars might both decrease the RKN population density and yield better in RKN-infested areas, when compared to susceptible genotypes. In Brazil, no cotton cultivars showing both resistance to RKN and good agronomic characteristics are available. Therefore, identifying and characterizing sources of resistance that can be incorporated into improved materials are the key to a future sustainable cotton production (Alves et al. 2017).

In general, the process of screening for RKN-resistant genotypes presents a high variation, due to the intrinsic characteristics of the host-nematode interaction. Anava has been widely used in plant breeding, but there are several limitations to this approach, such as the assumption of independence from errors and analyses of unbalanced data resulting from loss of plots or non-orthogonal experimental designs (Freitas et al. 2013). Thus, efficient selection tools are necessary to accurately identify potential genetic resistant sources. In this sense, the REML/Blup methodology is promising, because it estimates genotypic values, ensuring a greater accuracy in the selection process (Resende 2007). However, mixed models have not yet been used for the screening of RKN-resistant cotton genotypes. Thus, this study aimed to select cotton genotypes resistant to *Meloidogyne incognita* race 3, using REML/Blup.

## MATERIAL AND METHODS

The experiment was carried out in a greenhouse (21°14'05''S, 48°17'09''W and altitude of 614 m), from October 2015 to January 2016. The climate is Aw, with transition to Cwa. The experiment was completely randomized, with 32 cotton genotypes (Table 1) and seven replications. One plant inoculated with *M. incognita* race 3 per pot was considered as one replicate. The cotton cultivars 'FM 966' and 'M315-RNR' were used as susceptibility and resistance controls, respectively.

The confirmation of the inoculum identity was carried out using a photonic microscope, perineal pattern morphology (Taylor & Netscher 1974), male lips morphology (Eisenback & Hirschmann 1980) and esterase pattern (Esbenshade & Triantaphyllou 1990).

Table 1. List of cotton accessions (origin: Embrapa Algodão) inoculated with 5,000 eggs and second-stage juveniles of *Meloidogyne incognita* race 3.

Accession	Scientific name
M315-RNR	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
FM966	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
AUB 612 RNR	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
BJ 3128	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
DELTAPINE	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
COODETEC 404	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
DUNN 224	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
BRS 814	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
STONEVILLE C.	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
PARROT	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
CNPA-9615	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
4959	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
ALLEN 3357	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
IAC 25	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
ACALA 22	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
WILD M. J. JONES	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
CS8601	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
MCU5	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
SA2572	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
CE0415	<i>Gossypium barbadense</i>
AP 0460	<i>Gossypium hirsutum</i> var. <i>marie galante</i> mocó
RR0458	<i>Gossypium barbadense</i>
PI 0430	<i>Gossypium barbadense</i>
PA04158	<i>Gossypium hirsutum</i> var. <i>marie galante</i> mocó
IAPAR 97-141	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
PE 09-441	<i>Gossypium hirsutum</i> var. <i>marie galante</i> mocó
PIMA 56	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
PA0419	<i>Gossypium hirsutum</i> var. <i>marie galante</i> mocó
MT 03-185	<i>Gossypium hirsutum</i> var. <i>marie galante</i> mocó
ULTRAPRECOCE	<i>Gossypium hirsutum</i> var. <i>latifolium</i>
GO 04-05	<i>Gossypium hirsutum</i> var. <i>marie galante</i> mocó
MT 03-03	<i>Gossypium hirsutum</i> var. <i>marie galante</i> mocó

To evaluate the isoenzymatic phenotype for esterase, the BIO-RAD Mini Protean II vertical electrophoresis system was used. The subpopulations of *M. incognita* race 3 were multiplied in 'Santa Cruz Kada' tomatoes. Plastic pots were filled with a mixture of soil, sand and tanned bovine manure at a 3:1:1 ratio. This mixture was pre-autoclaved at 120 °C and 1 atm for one hour. After 90 days of inoculation, the inoculum used in the experiment was prepared according to Hussey & Barker (1973). The populations of eggs and second-stage juveniles ( $J_2$ ) were estimated using a photonic microscope and a Peters chamber.

The seedlings were produced in 128-cell expanded polystyrene trays filled with commercial coconut-based substrate. After sowing, the trays were conditioned in a greenhouse equipped with

a sprinkler irrigation system. The seedlings were transplanted at 15 days after sowing to 5-L plastic pots. The pots were filled with the autoclaved mixture previously described and placed in a greenhouse. The inoculation of 5,000 eggs and  $J_2$  was performed at transplanting, this being the initial population (Ip).

The nematodes were extracted according to Hussey & Barker (1973), at 90 days after the inoculation. Before the extraction, the roots were weighed using a digital scale. The final population (Fp) of eggs and  $J_2$  was estimated using a photonic microscope and a Peters chamber. Based on the Ip and Fp, the reproduction factor (RF) ( $RF = Fp/Ip$ ) was estimated. The genotypes were classified according to Oostenbrink (1966), who stated that materials with  $RF < 1$  are resistant and  $RF \geq 1$  are susceptible. The reproduction index (RI), based on the Fp of the susceptible control 'FM 966' ( $RI = Fp$  of a given genotype/Fp 'FM 966' \* 100), was also estimated. The genotypes were classified according to Taylor (1967), with  $RI > 51$  % being susceptible, between 26 and 50 % slightly resistant, between 11 and 25 % moderately resistant, between 1 and 10 % very resistant, and  $RI < 1$  % highly resistant or immune.

The SELEGEN-REML/Blup software was used for statistical analysis (Resende 2016). The statistical model 83 (completely randomized) was used:  $y = Xu + Zg + e$ , where  $y$  is the vector of data;  $u$  the scalar for the general mean (assumed as fixed);  $g$  the vector of genotypic effects (assumed as random);  $e$  the vector of errors or residuals (random); and  $X$  and  $Z$  the incidence matrices for  $u$  and  $g$ , respectively.

## RESULTS AND DISCUSSION

The cotton genotypes were submitted to infection by *M. incognita* race 3 in order to identify potential resistant accessions using REML/Blup. The nematode identity was confirmed using the perineal pattern morphology, male lips morphology and esterase pattern. The final population was measured and the reproduction factor and index estimated. The final population and reproduction index presented the highest values for genotypic variance ( $V_g$ ) and genetic variation coefficient ( $CV_g$ ) (Table 2), indicating a favorable situation for a breeding program aiming to develop RKN-resistant cotton genotypes. However, crosses between resistant and good-yielding genotypes could likely broaden the genetic variability and increase the likelihood

Table 2. Estimates of genetic parameters for the final population (FP) of root-knot nematodes, reproduction factor (RF) and reproduction index (RI) obtained in evaluations of cotton accessions inoculated with 5,000 eggs and second-stage juveniles of *Meloidogyne incognita* race 3.

Source of variation	FP	RF	RI
$V_e$	12,226.69	2.0113	7.6610
$V_g$	11,656.16	1.7145	6.8949
$V_f^g$	23,882.85	3.7276	14.5551
$h^2_g$	48.81	46.0429	47.3654
$h^2_{mg}$	86.97	85.6595	86.2999
$A_{cgen}$	93.26	92.5524	92.8977
$CV_g$	89.93	59.1220	73.2894
$CV_e$	92.10	64.0017	77.2586
$CV_g/CV_e$ ratio	0.98	0.9238	0.9486
General mean	120.06	2.2159	3.5826

$V_e$ : residual variance;  $V_g$ : genotypic variance;  $V_f$ : individual phenotypic variance;  $h^2_g$ : broad-sense heritability for individual plots;  $h^2_{mg}$ : genotype mean heritability;  $A_{cgen}$ : accuracy of genotype selection;  $CV_g$ : coefficient of genotypic variation;  $CV_e$ : coefficient of residual variation.

of selection success, as genetic variability is a basic condition for obtaining gains from selection (Falconer 1987, Cruz & Regazzi 2006).

For all sources of variation, the coefficient of individual heritability ( $h^2_g$ ) was below 50 % (Table 2). Resende (2002) reported that low  $h^2_g$  values are common for quantitative traits and indicate moderate to high heritability indices at the progeny level. The genotype mean heritability ( $h^2_{mg}$ ) was higher than 85 % for final population, reproduction factor and reproduction index (Table 2). We argue that selection based on individuals would be difficult, as there is a significant influence of the environment on nematode genotype and reproduction. However, genotype mean-based selection would be efficient. Alves et al. (2017) reported that the genetic control of cotton resistance to *Meloidogyne incognita* race 3 is explained as an oligogenic inheritance. The resistance may suffer a greater influence from the environment, if compared to monogenic inheritance. The authors also claim that polygenic and oligogenic resistances are interesting and necessary tools for managing plant diseases. Although oligogenic inheritance results in different resistance levels, from a high susceptibility to a high resistance, they are more stable than monogenic resistance.

$CV_e$  is an unsuitable parameter to evaluate the quality of experiments, as it does not take into account the level of genotypic variation and the number of replicates. Accuracy of selection is more appropriate for measuring experimental accuracy (Resende & Duarte 2007), as it includes  $CV_e$ ,  $CV_g$  and the number

of replicates. The accuracy of selection was very high for final population, reproduction factor and reproduction index according to Resende & Duarte (2007), who established that values above 70 % are considered high. High accuracy values indicate a high correlation between predicted and real genotypic values (Torres Filho et al. 2017). Therefore, the accuracies obtained ranged from high to very high and indicate the possibility of an effective selection.

The genotypic variation coefficient (CVg) *per se* is not very representative; however, the CVg/CVe ratio indicates to a certain extent whether the phenotype selection of the characteristic will be efficient. Therefore, when the relation is greater than the unit, it is feasible to select a given character (Vencovsky & Barriga 1992). For all characters, the CVg/CVe values were lower than 1, indicating a

greater environmental variation than the genotypic one (Table 2). Thus, only phenotype-based selection could not achieve the genotype desired by the breeder.

The 32 genotypes in the present study were evaluated based on the genotypic effects (g) and the general mean plus genotypic value (u + g). The values referring to the new mean are the predictions provided by Blup for commercial cropping; these cotton genotypes should result, on average, in such values.

If selection is to be performed based on the RKN reproduction features, the lowest values are the goal, as they mean less nematode development and reproduction. In this sense, the genotypes CS8601, SA2572, Coodetec 404 and BJ 3128 showed the lowest values for all characteristics, thus being good genetic resistance sources that can be used in cotton breeding programs (Table 3).

Table 3. Genotypic effects (g), general mean plus genotypic values (u + g) and raking (Rk) of cotton accessions inoculated with 5,000 eggs and second-stage juveniles of *Meloidogyne incognita* race 3.

Genotypes	Final population				Reproduction factor				Reproduction index						
	Rk	g	u + g	Gain	Na	Rk	g	u + g	Gain	Na	Rk	g	u + g	Gain	Na
CS8601	32	-28,661.0	9,215.75	0	37,876.3	32	-5.732	1.843	0	7.575	32	-19.780	6.360	0	26.139
SA2572	31	-28,465.0	9,411.66	924.54	38,800.9	31	-5.693	1.882	0.185	7.760	31	-19.645	6.495	0.649	26.788
BJ 3128	30	-28,465.0	9,411.66	1,904.18	39,780.5	30	-5.693	1.882	0.381	7.956	30	-19.645	6.495	1.293	27.432
COODETEC 404	29	-28,269.0	9,607.56	2,951.38	40,827.7	29	-5.654	1.922	0.590	8.166	29	-19.509	6.631	2.037	28.176
AP 0460	28	-28,073.0	9,803.47	4,066.38	41,942.7	28	-5.615	1.961	0.813	8.389	28	-19.375	6.765	2.857	28.997
AUB 612 RNR	27	-28,073.0	9,803.47	5,256.73	43,133.1	27	-5.615	1.961	1.051	8.627	27	-19.375	6.765	3.562	29.701
WILD M. J. JONES	26	-27,681.0	10,195.30	6,538.63	44,415.0	26	-5.536	2.039	1.308	8.883	26	-19.103	7.037	4.513	30.652
ACALA 22	25	-27,485.0	10,391.20	7,907.42	45,783.8	25	-5.497	2.078	1.582	9.157	24	-18.969	7.170	6.475	32.614
M315-RNR	24	-27,485.0	10,391.20	9,382.11	47,258.5	24	-5.497	2.078	1.876	9.452	25	-18.970	7.169	5.457	31.596
PARROT	23	-27,289.0	10,587.10	10,985.00	48,861.4	23	-5.458	2.117	2.197	9.772	23	-18.833	7.307	7.581	33.720
IAPAR 97-141	22	-27,093.0	10,783.00	12,724.80	50,601.1	22	-5.419	2.157	2.545	10.120	22	-18.698	7.441	8.782	34.921
PA 04-158	21	-26,310.0	11,566.60	14,620.90	52,497.2	21	-5.262	2.313	2.924	10.499	19	-18.157	7.982	13.064	39.203
IAC 25	20	-26,310.0	11,566.60	16,667.40	54,543.7	20	-5.262	2.313	3.334	10.909	20	-18.158	7.981	11.503	37.642
DUNN 24	19	-26,310.0	11,566.60	18,929.40	56,805.7	19	-5.262	2.313	3.786	11.361	21	-18.158	7.981	10.090	36.230
STONEVILLE C.	18	-24,938.0	12,938.00	21,442.60	59,319.0	18	-4.988	2.588	4.289	11.864	18	-17.210	8.929	14.798	40.938
MCU5	17	-22,196.0	15,680.70	24,170.90	62,047.3	17	-4.439	3.136	4.834	12.410	17	-15.317	10.822	16.681	42.820
ALLEN 3357	16	-21,020.0	16,856.10	27,068.90	64,945.2	16	-4.204	3.371	5.414	12.989	16	-14.508	11.632	18.681	44.820
4959	15	-20,432.0	17,443.80	30,274.80	68,151.1	15	-4.087	3.489	6.055	13.630	15	-14.100	12.039	20.894	47.033
BRS 814	14	-17,494.0	20,382.50	33,896.70	71,773.1	14	-3.499	4.077	6.779	14.355	14	-12.074	14.065	23.393	49.532
CE0415	13	-16,318.0	21,557.90	37,849.90	75,726.2	13	-3.264	4.312	7.570	15.145	13	-11.262	14.877	26.122	52.261
CNPA-9615	12	-12,204.0	25,671.90	42,363.90	80,240.2	12	-2.441	5.134	8.473	16.048	12	-8.422	17.717	29.237	55.376
PE 09-441	11	-9,461.7	28,414.60	47,324.60	85,201.0	11	-1.892	5.683	9.465	17.040	11	-6.530	19.610	32.660	58.800
MT 303	10	4,447.7	42,324.04	53,003.30	90,879.6	10	0.890	8.465	10.601	18.176	10	3.070	29.209	36.579	62.719
DELTAPINE	9	16,790.0	54,666.20	58,398.30	96,274.7	9	3.358	10.933	11.680	19.255	9	11.588	37.727	40.303	66.442
RR0458	8	26,193.0	64,069.70	63,599.40	101,475.7	8	5.239	12.814	12.720	20.295	8	18.076	44.216	43.892	70.031
ULTRAPRECOCE	7	28,740.0	66,616.50	68,943.10	106,819.4	7	5.748	13.323	13.789	21.364	7	19.834	45.973	47.580	73.719
GO 0405	6	49,310.0	87,186.80	75,643.60	113,519.9	6	9.862	17.437	15.129	22.704	6	34.031	60.170	52.204	78.343
PI 0430	5	53,424.0	91,300.80	80,910.20	118,786.6	5	10.685	18.260	16.182	23.757	5	36.871	63.010	55.839	81.978
PA0419	4	64,003.0	101,880.00	87,781.70	125,658.0	4	12.801	20.376	17.556	25.132	4	44.171	70.310	60.581	86.720
FM966	3	81,537.0	119,413.00	95,707.70	133,584.1	3	16.307	23.883	19.142	26.717	3	56.271	82.410	66.051	92.190
MT 03-185	2	89,471.0	127,348.00	102,793.00	140,669.4	2	17.894	25.470	20.559	28.134	2	61.748	87.887	70.941	97.080
PINA 56	1	116,115.0	153,991.00	116,115.00	153,991.1	1	23.223	30.798	23.223	30.798	1	80.135	106.274	80.135	106.274

Na: new average.



The ability of the selected genotypes to perform satisfactorily in RKN-infested areas in various environments and their resistance predictably behavior resulting from their genetics and environmental improvements are goals to be achieved. This shows the relevance of studies on mixed models for cotton. Furthermore, mixed models are adequate to select resistant genotypes in RKN-infested open field trials considering that some specimens may be highly susceptible and may fail to sufficiently develop. In such cases, evaluations can be performed, but may cause an experimental misbalance (Salgado et al. 2014).

### CONCLUSION

The genotypes CS8601, SA2572, Coodetec 404 and BJ 3128 are promising for cotton breeding programs aiming the selection of cotton genotypes resistant to root-knot nematodes.

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