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Rpp gene inheritance in soybean (*Glycine max* (L.) Merrill) for resistance to Asian rust

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ABSTRACT

The four donors, SDP10 (Rpp 1, & Rpp 3), SDP18 (Rpp 2), SDP30 (Rpp2), and SDP36 (Rpp2), were crossed with the common susceptible female JS335 line of soybean. The goal of this experiment was to determine the inheritance of rust-resistant Rpp genes (Rpp1, Rpp 2, Rpp3) against Asian soybean rust. Because the F_1 plants from the four crossings were resistant to rust, as researchers concluded that dominant genes are in charge of the rust resistance. The JS335 x SDP10 plants of the F_2 generation were segregated in a ratio of 15 resistant: 1 susceptible and the BC_1 F_1 plants were separated in a ratio of 3 resistant: 1 susceptible, indicating the presence of duplicate gene interaction. The F_2 offspring of the crosses JS335 x SDP18, JS335 x SDP30, and JS335 x SDP36 segregated in a 3 resistant: 1 susceptible ratio. However, in backcross (BC_1 F_1) generations, the test cross under the investigation was segregated in a 1 resistant: 1 susceptible ratio, suggesting that in these crosses, soybean rust resistance was governed by a dominant gene.

Keywords- *Soybean, Asian Rust, PDI, AUPDC, Rpp genes.*

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INTRODUCTION

The area of soybean (*Glycine max* (L.) Merrill) farming worldwide is 127 million hectares, with a yield of 364.33 million metric tons in the year 2018-2019. The world's largest soybean producer is the United States. Other significant producers are China (7%) with 16 million metric tons, Argentina (18%) with 53.50 million metric tons, Brazil (31%) with 117.80 million metric tons, and India (4%) with 12.10 million metric tonnes garins production. Leaf rust (*Phakopsora pachyrhizi*), is one of the most harmful foliar disease of soybeans globally, is a one kind of fungus. In fields that are not protected from infestation, losses of up to 75% have been recorded [1]. For sustainable soybean production, breeding for biotic and abiotic stress resistance is preferred since it reduces environmental impact and cultivation expenses. It is vital to find and create superior stress-tolerant soybean lines that can be used to create genetically superior kinds in order to lessen losses from biotic and abiotic challenges.Therefore, regulating genetic resistance is both strategically and economically vital for controlling soybean rust disease [2]..

Seven *Rpp* genes and three alleles for pathotype-specific resistance to soybean rust (*Rpp*) have been identified including *Rpp1* from PI 200492[3], *Rpp2* from PI [4], *Rpp3* from PI 462312 (Ankur) [5,6,7], *Rpp4* from PI 459025B [8], *Rpp4b* from PI 423972 [9]; *Rpp5* from PI 200456 [10], *Rpp6* from PI 567102B [11], *Rpp1-b* (another allele at the *Rpp1* locus) from PI 594538A [12] and *Rpp?* (*Hyuuga*) (An allele at the *Rpp3* locus) from the Japanese cultivar *Hyuuga*, designated PI 506764 [13]. *Rpp7* was recently discovered in PI 605823 based on its resistance to *P. pachyrhizi* [14]. These genes are located at various loci in the distinct genotype of several races of pathogens. Because *Phakopsora pachyrhizi* has high genetic diversity, developing soybean cultivars resistant to leaf rust could prove challenging. Monogenic resistance is unlikely to give long-lasting protection. It is helpful for plant breeders to choose an appropriate breeding strategy for enhancing an existing line and the choice of parental material since the information on gene action provides interpretation for the regulation of inheritance for rust resistance.

MATERIALS AND METHODS

Plant material and crossing programme

The double cross hybrid (PI 200492-Komata PI 230971) (PI 462312-Ankur PI 459025-Bing Nan) developed by crossing four different resistance sources, each having a single gene *Rpp1, Rpp2, Rpp3,* and *Rpp4* was used to produce four rust-resistant donor parents (males) for the current study[15,16 &17]. At the Agriculture Research Station (ARS), Kasbe Digraj, Sangli, Maharashtra, India the segregating generations with combinations of various *Rpp* genes were further assessed up to the F_5 generation under hotspot rust screening. Additionally, F_6 generation seeds were planted in Kharif in 2017 at Kasbe Digraj Hot Spot Agricultural Research Station and tested for rust screening. During Kharif 2017, resistant lines SDP10, SDP18, SDP30, and SDP36 were found and coded. They were then crossed independently with the common female JS335 to get the results. Resistance is present in male parents SDP10 (*Rpp1* & *Rpp3*), SDP18 (*Rpp2*), SDP30 (*Rpp2*), and SDP36 (*Rpp2*), as well as the original *Rpp* donors, were found to have *Rpp* genes. (by employing linked molecular markers, checks PI 200492-Komata *Rpp1,* PI 230971(*Rpp2*), PI 462312-Ankur (*Rpp3*), and PI 459025-Bing Nan-*Rpp4*). During the summer of 2018, confirmed F_1 were selfed and backcrossed with parent JS335 to produce F_2 and backcrosses (BC_1 F_1). In the Kharif of 2018, the Agricultural Research Station in Kasbe Digraj, District-Sangli (field condition), a hot spot for the occurrence of rust assigned ideal conditions, conducted a randomised block design with three replications for the rust screening of P_1, P_2, F_1, F_2, and backcross (BC_1 F_1), of four crosses, four original Rpp gene donors (Checks). The experiment was planted on July 25, 2018 (late seeded), when disease development could be the most rapid. The sowing procedure involved using 3 m long rows with a 45 x 10 cm spacing between each plant and row. P_1, P_2, and F_1s each received one row, while backcross (BC_1 F_1) received two rows. F_2s received 10 rows. This has allowed for the growth of 45 plants in each of the P_1s, P_2s, and F_1s, 300 plants in each of the F_2, and 60 plants in the backcross (BC_1 F_1), with one row set aside for each check and 15 plants of each check-in three replications. For the irrigated scenario, a fertilizer dose of 50 kg N and 75 kg P_2O_5/ha was used at the time of sowing. An aqueous suspension of rust spores was sprayed over the test subject to ensure uniform disease transmission. The illness first manifested in the first week of September 2018. For the rust screening, observations on rust intensity and sporulation were recorded on 40 plants from parents, F_1s and original Rpp source (Checks), 200 to 300 plants from F_2s, and 20 to 30 plants from Backcross (BC_1 F_1), generations in each replication. Initially, ash to TAN-coloured pustules was seen on the susceptible female JS335, and later, the disease spread to the entire field. According to rust pustule colour (sporulation), plants were categorized as resistant (R) or susceptible (S) for rust scoring. Immune (no sporulation) indicates complete resistance, Reddish-brown (RB) lesions indicate incomplete resistance, and profusely sporulation tan lesions indicate susceptibility. The Mayee and Datar [18], 0–9 scale was used to measure the severity of the rust-pustule infection. The scale reads 0 = resistant reaction with 0% disease intensity, 1 = highly resistant reaction with 1% disease intensity, 3 = moderately resistant with 1.1–10% disease intensity, 5 = moderately susceptible reaction with 10.1-25% disease intensity, 7 = susceptible reaction with 25.1–50% disease intensity, and 9 = highly susceptible reaction with more than 50% disease intensity. Using the percent Disease Index (PDI), [19, 20 &15] investigated soybean rust resistance. The following formula was used to compute the percent illness intensity and the area under the disease progression curv.

Percent Disease Intensity (PDI)

$$\text{P.D.I.} = \frac{\text{Sum of all numerical ratings}}{\text{Number of leaves observed} \times \text{Maximum disease grade}} \times 100$$

The Area Under Disease Progress Curve (AUDPC)

In order to grade the host resistance, observations are taken at weekly intervals beginning with the first sign of rust. The five observations (49th, 56th, 63rd, 70th, and 79th DAS) were all recorded to determine the AUDPC values. For each genotype, the area under the illness progress curve was calculated using the equation below [21].

$$\text{AUDPC} = ½ \sum_{i=1}^{K} \{Si + S\,(i-1)\,d\}$$

RESULTS AND DISCUSSION

The inheritance of *Rpp* genes was mentioned in the table 1, and the genetics of soybean rust resistance as seen in the current discovery is presented cross-wise. In tables 2 and 3, the percentage of disease intensity (PDI) and the area under the disease progression curve were displayed. According to the findings of the current inquiry, 39 plants were all resistant and had immunological (no sporulation) type of hypersensitive reaction (total resistance), and PI 200492-Komata is the source of validated rust-resistant *Rpp1* gene. Another line PI 230971 is source of Rpp2 gene, all 39 plants of it showed Reddish-brown (RB) lesions (incomplete resistance), Rpp3 gene is Indian origin and found in PI 462312-Ankur having issue of partial breakdown, out of 39 plants of PI 462312- Ankur 27 showed Reddish-brown (RB) lesions (incomplete resistance) and 12 plants were showed profusely sporulating tan lesions (susceptible). All plants from the line PI 459025-Bing Nan, a verified source of the *Rpp4* gene, had reddish-brown (RB) lesions, while all 39 plants from the female parent JS335 displayed profusely sporulating tan lesions (susceptible). All plants from male parents were hardy. All 39 plants of the other three donor male SDP18 (*Rpp2*), SDP30 (*Rpp2*), and SDP36 *(Rpp2*) had resistant plants with Reddish-brown (RB) lesions (incomplete resistance), out of the 39 plants of SDP10 (*Rpp1,* & *Rpp3*), 15 plants showed Immune and 24 were RB type rust resistant reaction.

All rust-resistant (Immune/RB) plants from the JS335 x SDP10 cross were produced in the F_1 generation. It concludes that a dominant gene regulated the resistance. Out of the 275 plants examined in the F_2 generation, 253 had rust resistance (Immune/RB) and 22 had rust susceptibility (TAN). It was evidently non-significant, with a Chi-square score of 1.38. In the research of the Backcross (BC_1 F_1) generation, which was the test cross analyzed for 28 plants, it was found that the observed ratio of 14.72:1.28 nearly matched the fitting table 15:1, indicating the presence of duplicate gene interaction. The data's Chi-square value was non-significant (1.40). The observed ratio of 2.58 to 1.42 roughly matched the fitting table's 3:1 recommendation. For the inheritance of both *Rpp1* and *Rpp3* in soybean leaf rust, the test cross and F_2 ratios provided conclusive evidence of the existence of duplicate gene interaction. One allele is sufficient to provide rust resistance in this duplicate gene interaction, or the presence of either dominant gene ensures rust resistance. Similar duplicate gene interactions have been observed [22, 23 &15] for the inheritance of rust resistance.

In the second cross, JS335 x SDP18 (*Rpp2*), every plant from the F_1 generation that was rust-resistant showed a reddish-brown rust reaction. Of the 286 plants from the F_2 generation that were analyzed, 213 of them were rust-resistant (RB) and 73 were rust-susceptible (TAN) plants. The Chi-square value of the data was non-significant (0.123). In the studied backcross (BC_1 F_1) generation for 24 plants, out of which 14 were rust resistant and 10 were rust susceptible, the observed ratio of 2.98:1.02 was closely fitted with the fitment table 3:1 to indicate the presence of monogenic gene interaction. It also clearly displayed a non-significant Chi-square value (0.26). The observed ratio of 1.17:0.83 was closely fitted with the fitment table 1:1. The test cross and F_2 ratios supported the hypothesis that the resistance to rust is controlled by a single *Rpp2* gene. The third cross between JS335 and SDP30 (*Rpp2*) resulted in F_1 generation plants that were all rust-resistant (RB). The observed ratio of 3.14:0.86 closely fitted with the fitment table 3:1 indicating presence of monogenic gene interaction for the inheritance of monogenic Rpp2 gene in the segregating F_2 populations, out of the 260 plants studied, 186 were rust resistant (RB) and 74 were rust susceptible (TAN) plants. In the studied backcross (BC_1 F_1) generation, out of 28 segregated plants, 16 were rust resistant and 12 were rusting susceptible having non-significant Chi-square values. The observed ratio was 1.15: 0.85 closely fitted with the fitment table 1:1. These test cross ratios demonstrate that the cross in question inherits a single dominant gene.

All plants of the F_1 generation in the fourth cross between JS335 and SDP36 (Rpp2) showed rust resistance with RB type of rust reaction, which limits the growth of pathogens. This resistance is controlled by *Rpp2* genes with dominant expression. The 280 plants in the F_2 generation were divided into 206 rust-resistant (RB) and 74 rust-susceptible (TAN) plants. The Chi-square result (0.29), however, was not noteworthy. Because of how well the measured ratio of 2.95:1.05 matched the fitting table 3:1, monogenic gene interaction was present. Out of the 24 plants studied in backcross (BC_1 F_1), 11 were resistant to rust and 13 were sensitive, with a non-significant Chi-square value (0.16), The measured ratio of 1.08:0.92 was well-fitted by the fitting table of 1:1 at that value. The test cross ratio verified the existence of a monogenic gene interaction for the transmission of soybean leaf rust.

The F_1 of four crosses that were resistant to soybean rust showed that the resistance in these crosses was driven by dominant genes. One dominant gene is involved in the inheritance of soybean rust in this monogenic interaction. In these three crossings, the F_2 offspring were segregated into 3 resistant: 1 susceptible ratio. One dominant gene that participated in this monogenic interaction was responsible for the inheritance of soybean rust. This finding is in line with earlier research [24, 22, 23, 25, 26 & 20], which found that rust resistance in soybeans is controlled by a dominant gene and that 3:1 monogenic inheritance was observed in F_2 generations. 3:1 F_2 ratio was reached in a cross with resistance dominating [3].

The area under the disease curve and the percent disease intensity

In contrast to the male donor SDP10, who recorded a PDI value of 7.77% with AUDPC (24.40), which indicated immune and RB reaction to rust, the common female JS335 had a PDI value of 97.77% with AUDPC (1073.65) that showed densely TAN coloured patches covered into both sides of the leaves that were highly susceptible to rust (Table 2 and 3).

The PDI value recorded in common female JS335 was 97.77 percent with AUDPC (1073.65) which showed densely TAN-colored patches covered into both sides of leaves which highly susceptible to rust, while male donor SDP10 recorded 7.77 percent PDI with AUDPC (24.40) which showed immune and RB reaction to rust.

The F_1 further showed both immune to rust and reddish brown to rust reactions with 10.0% PDI with AUDPC (27.0). With AUDPC (620.0 and 247.99), the illness intensity in the F_2 and backcross (BC_1 F_1) generations was 66.66% and 33.33%, respectively. Male donor SDP18 recorded a 13.13% PDI with a resistance-indicating AUDPC (40.69) value. The illness intensity was 16.16% in the JS335 x SDP18 F_1, and AUDPC (64.72%) displayed an insufficient RB resistance response to rust. The F_2 had a PDI value of 51.11 percent and AUDPC (460.66), but the backcross (BC_1 F_1) had a PDI value of 81.11 percent and AUDPC (816.16), respectively.

Early maturing male donor SDP30 exhibited a PDI of 11.11% with AUDPC (31.66) which revealed RB sensitivity to rust. The illness intensity in the F_1 of JS335 x SDP30 was 15.15%, and AUDPC (60.24) displayed an insufficient RB resistance response to rust. The F_2 had a PDI value of 72.22 percent with AUDPC (698.65), whereas the B_1 had a PDI value of 46.66 percent with AUDPC (371.49). SDP36 revealed a 20% PDI with AUDPC (72.00), indicating RB rust response. The illness intensity in the F_1 of JS335 x SDP36 was 22.22 percent, and AUDPC (90.33) displayed an insufficient RB-resistant response to rust. The F_2 had a PDI value of 77.77%, whereas Backcross (BC_1 F_1) had a PDI value of 84.44%, with AUDPC (735.33 and 807.99), respectively.

Although using resistance genes offers a chance to control disease in soybeans, their "race-specific" nature could present issues [27 & 28]. According to Tschanz *et al*. [29], the soybean-resistant lines TK-5, Tainung-4, and PI239871A may all share a single dominant gene for resistance. According to Tan *et al*. [30], resistance in the PI459025 line was managed by a dominant gene, whereas in the cultivars AGS 129 and AGS 181, it was managed by a number of genes. Rust resistance is controlled by a single dominant gene, according to an F_2 segregation analysis of six susceptible x resistant cross combinations [22]. In *G. tomentella,* the resistance was controlled by a single dominant gene in aneuploids (2n = 78) and by two or three gene loci in in tetraploids (2n=80) [31]. BR 01-18437 was controlled by a single recessive major gene, whichwas also distinct from *Rpp1* through *Rpp4* and different from the genes in PI 200487 and PI 200526 [32].

The resistance line PI 197182, PI 230971, and PI 417125 genotypes each have a single resistance gene in the Rpp2 locus. Seven soybean genotypes were found to be resistant to rust [33]. These genotypes were TG x 1987-62F, TG x1935-3F, TG x 1951-3F, TG x 1936-2F, TG x 1987-10F, TG x 1972-1F, and TG x 1949-8F. The mode of inheritance revealed that rust resistance in soybean was monogenically controlled by dominant genes. Plant introduction (PI) 561356's SBR resistance was mapped [34]. The population's segregation ratio between reddish brown and tan lesion types corroborated the finding that a single dominant gene was responsible for controlling resistance. When Li *et al*. [11] analyzed data from two distinct populations, they discovered that PI 567102B's resistance was driven by a single dominant gene known as *Rpp6.*[35] found that soybean plants with two gene combinations (homozygous dominant or heterozygous at both loci) showed considerably less disease severity and sporulation in the F_2 generation, indicating complementary epistatic gene action for resistance. Gene *Rpp3* contributed positively to resistance with various genetic backgrounds for most parameters measured, compared to *Rpp2* and *Rpp4* resistance genes. The rust resistance genes in soybean rust were reviewed by Bhor *et al*. (2014 [20]). Rust resistance inheritance is typically governed by a single dominant gene, occasionally by two dominant and one recessive gene, and very rarely by two and three recessive genes. When Matsuo *et al.* [26] examined the inheritance of the *Phakopsora pachyrhizi* resistance gene in soybean cultivar TMG 803, they discovered that the resistance was regulated by a single gene that had complete dominance and was designated as resistance locus Rpp4. Using the segregating F_2 generations of the cross-A (PI 200492 x PI 230971) and cross-B (PI 462312 x PI 459025), Parhe *et al*. [15] studied the inheritance of rust resistance from pyramided generations to four soybean accessions PI 200492

(Komata), PI 230971, PI 462312 (Ankur), and PI 459025 (Bing Nan). The F_2 progenies from cross A separated into 13 resistant and 3 susceptible individuals, demonstrating redundant gene interactions. In cross-B, the F_2 progenies are segregated in a 3 resistant: 1 susceptible ratio, demonstrating that soybean rust resistance is passed down monogenetically and dominantly.

CONCLUSION

The purpose of this study was to examine the inheritance of Rpp genes (Rpp1, Rpp 2, and Rpp3) that are resistant to Asian soybean rust. As determined in the experiment that dominant genes are responsible for rust resistance, it was found in this experiment that the F_1 plants from the four crossings were resistant to rust. The BC_1 F_1 plants were divided in a ratio of 3 resistant: 1 susceptible, however, the JS335 x SDP10 plants of the F_2 generation were segregated in a ratio of 15 resistant: 1 susceptible, showing the presence of duplicate gene interaction. The F_2 offspring of the crosses JS335 x SDP18, JS335 x SDP30, and JS335 x SDP36 segregated in a 3 resistant: 1 susceptible ratio, suggesting that soybean rust resistance is passed down monogenetically and dominantly.

Table 1: Summarized data on inheritance of rust resistance under field condition in all crosses

Crosses	Environ-ment	Gene-Rations	Number of plants observed			Expected Ratio		Observed Ratio		Number of plants expected		χ^2	P value	Gene action
			R	S	Total	R	S	R	S	R	S			
Cross-I JS335 x SDP10	Field Condition	P_1	0	39	39	-	-	-	-	-	-	-	-	-
		P_2	39	0	39	-	-	-	-	-	-	-	-	-
		F_1	24	0	24	-	-	-	-	-	-	-	-	-
		F_2	253	22	275	15	1	14.72	1.28	257.82	17.18	1.38 (N.S.)	0.24	Duplicate
		B_1	18	10	28	3	1	2.58	1.42	21	7	1.40 (N.S.)	0.23	Duplicate
Cross-II JS335 x SDP18	Field Condition	P_1	0	39	39	-	-	-	-	-	-	-	-	-
		P_2	39	0	39	-	-	-	-	-	-	-	-	-
		F_1	25	0	25	-	-	-	-			-	-	-
		F_2	213	73	286	3	1	2.98	1.02	214.5	71.5	0.12(N.S.)	0.28	Monogenic
		B_1	14	10	24	1	1	1.17	0.83	12	12	0.26(N.S.)	0.61	Monogenic
Cross-III JS335 x SDP 30	Field Condition	P_1	0	39	39	-	-	-	-	-	-	-		-
		P_2	39	0	39	-	-	-	-	-	-	-		-
		F_1	23	0	23	-	-	-	-	-	-	-		-
		F_2	186	74	260	3	1	3.14	0.86	195	65	1.52(N.S.)	0.21	Monogenic
		B_1	16	12	28	1	1	1.15	0.85	14	14	0.58 (N.S.)	0.44	Monogenic
Cross-IV JS335 x SDP 36	Field Condition	P_1	0	39	39	-	-	-	-	-	-	-	-	-
		P_2	39	0	39	-	-	-	-	-	-	-	-	-
		F_1	24	0	24	-	-	-	-	-	-	-	-	-
		F_2	206	74	280	3	1	2.95	1.05	210	70	0.29 (N.S.)	0.59	monogenic
		B_1	11	13	24	1	1	1.08	0.92	12	12	0.16 (N.S.)	0.68	Monogenic

R= Resistant S= Susceptible (X^2 table, at 5% = 3.8414 and at 1% = 6.634)

Table 2: Average PDI (Per cent Disease Intensity) valuesof different generations

Sr. No.	Name of cross	Environment	Generation	Percent disease Intensity	Conclusive reaction	Disease category
1.	Cross-I (S x R) JS335 x SDP10	Field Condition	P_1	97.77	TAN	Highly susceptible
			P_2	7.77	(RB+Immune)	Resistant
			F_1	10.0	(RB+Immune)	Resistant
			F_2	66.66	TAN	Highly susceptible
			B_1	33.33	TAN	Susceptible
2.	Cross-II (S x R) JS335 x SDP18	Field Condition	P_1	97.77	TAN	Highly susceptible
			P_2	13.13	(RB)	Resistant
			F_1	16.16	(RB)	Resistant
			F_2	51.11	TAN	Highly susceptible
			B_1	81.11	TAN	Highly susceptible
3.	Cross-III (S x R) JS335 x SDP30	Field Condition	P_1	97.77	TAN	Highly susceptible
			P_2	11.11	(RB)	Resistant
			F_1	15.15	(RB)	Resistant
			F_2	72.22	TAN	Highly susceptible
			B_1	46.66	TAN	susceptible

4.	Cross-IV (S x R) JS335 x SDP36	Field Condition	P_1	97.77	TAN	Highly susceptible
			P_2	20	(RB)	Resistant
			F_1	22.22	(RB)	Resistant
			F_2	77.77	TAN	Highly susceptible
			B_1	84.44	TAN	Highly susceptible
5	PI 200492	Field Condition	Check-1	15.15	(RB+Immune)	Resistant
6	PI 230971	Field Condition	Check-2	16.16	(RB)	Resistant
7	PI 462312	Field Condition	Check-3	24.44	(TAN)	Susceptible
8	PI 459025	Field Condition	Check-4	17.17	(RB)	Resistant

Table 3: Average AUDPC (Area under Disease Progress Curve) valuesof different generations

Sr. No.	Name of cross	Generations				
		P_1	P_2	F_1	F_2	B_1
1.	JS335 x SDP10	1073.65 (Localized TAN)	24.40 (RB + Immune)	27.00 (RB + Immune)	620.0 (Localized TAN)	247.99 (Localized TAN)
2.	JS335 x SDP18	1073.65 (Localized TAN)	40.69 (RB)	64.72 (RB)	460.66 (Localized TAN)	816.16 (Localized TAN)
3.	JS335 x SDP30	1073.65 (Localized TAN)	31.66 (RB)	60.24 (RB)	698.65 (Localized TAN)	371.49 (Localized TAN)
4.	JS335 x SDP36	1073.65 (Localized TAN)	72.00 (RB)	90.33 (RB)	735.33 (Localized TAN)	807.99 (Localized TAN)
5.	PI 200492	37.74 (RB + Immune)	-	-	-	-
6.	PI 230971	-	-	-	-	-
7.	PI 462312	46.75 (TAN)	-	-	-	-
8.	PI 459025	-	-	-	-	-

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