Minufiya J. Agric. Res. Vol. 27 No. 3: 595 - 606 (2002)

GENETIC STUDIES ON SOME EGYPTIAN AND WORLD WIDE COLLECTION OF RICE VARIETIES I-IDENTIFICATION OF NEW WIDE COMPATIBILITY VARIETIES

I. R. Aidy[2], H.A. Hamza[1], S.A. Salama[1] and A.F. Moustafa[2]
Egyptian Rice Research & Training Center (RRTC). Agricultural Research Center, Sakha, Egypt[2].
Genetics Department, Faculty of Agriculture, Minufiya University, Shibin El-Kom, Egypt[1].

(Received: Apr., 27 , 2002)

ABSTRACT: *Twenty-two Egyptian and World wide collection of rice varieties were used in this study. Crosses of nineteen of these varieties were performed with IR 36 as indica tester and Norin PL12 as japonica tester. Percentage of pollen viability and spikelet fertility expressed as seed set were scored in the progenies (F1s) in all crosses. Correlation between seed set and pollen viability in all obtained crosses were analyzed. Furthermore, the isozyme Amp3 was studied. Results showed that, the progenies (F1s) of the nineteen Egyptian and World wide collection varieties crossed with the indica tester (IR36) gave pollen viability values ranged from 14 to 90 % and ten out of the nineteen tested progenies gave more than 70% pollen viability. Meanwhile, the progenies obtained from japonica tester crossed with the nineteen studied varieties showed pollen viability values ranged from 25 to 90% and fourteen F1s showed more than 70% pollen viability. Spikelet fertility estimates of the F1s of crosses with the two testers showed that; in crosses with IR36 seven F1s out of nineteen gave more than 70 % seed set. In crosses with the Norin PL12 thirteen F1s showed more than 70 % seed set. Seed set showed to be more than 70% in the crosses with both indica and japonica testers for the varieties IR68022-50 and IR25571-31 in addition to the three known WCVs. Thus, the IR68022-50 tropical japonica variety and the IR25571-31 indica variety are recommended to be new WCVs. Correlation between pollen viability and seed set was stronger in crosses with indica tester than in crosses with japonica tester. Isozyme aminopeptidase-3 (Amp3) [E.C.3.4.11.1] studies of twenty-one varieties showed that, eleven varieties have Amp3-1 activity and ten have Amp3-2 activity. Six out of the latter ten varieties appeared to be WCVs.*

Key Words: Rice, Isozyme, Wide compatibility varieties, Spikelet fertility.

INTRODUCTION

In spite of the relative richness of the genetic variability in the rice gene pool, utilization of these germplasmes had been limited to only adaptable genotypes. Crosses between elite germplasmes proven for their outstanding

performance on the progenitor of superior cultivars. This contributed to the existence of a small number of genotypes being recycled, making modern rice varieties genetically related. Indica and japonica rices are genetically diverse, therefore indica/japonica hybrids could show strong heterosis for various traits, including total dry matter, tillers number, spikelet number and 1000-grain weight. However, heterosis for yield is difficult to be attained in these hybrids because of sterility, (Butany *et al.,* 1961 Jennings 1966 and Ikehashi 1982). Rice F1 hybrids between indica and japonica varietal groups exhibit varying degrees of pollen and spikelet sterility (Kato *et al.,* 1930; Butany *et al.,* 1961; Jenning 1966 and Yuan *et al.,* 1987). Previous studies revealed that the sterility in intervarietal crosses in rice was due to genetic differences (Terao and Mdusima, 1939; Sampath and Mohanty, 1954; Sampath 1963 and Ikehashi 1982). Several researchers have reported that certain rice varieties produce fertile F1 hybrids when crossed with indica or japonica lines (Terao and Mdusime, 1939; Jennings, 1966 and Heu, 1967). Such varieties were designated as wide compatible varieties (WCVs) (Ikehashi and Araki 1986). WCVs provide a bridging mechanism between indica and japonica varieties in rice improvement programs. The genetic basis of F1 sterility and WCVs in rice hybrids were due to multiple alleles locus (S5) located between C^+ (chromogen for apiculus colour) and Wx (waxy endosperm) loci in chromosome six (Ikehashi and Araki 1986). An interaction of two alleles out of three i.e., S5-i from indica and S5-j from japonica were found to confer female gamete abortion, which was expressed as semi-sterility of the panicle. Female gametes carrying S5-j were aborted in the genotype of S5-i / S5-j, while a neutral allele S5-n did not cause the abortion in heterozygous of S5-i /S5-n and S5-j /S5-n. The donor parent of S5-n was termed as wide compatible variety (WCV), Where S5-n is the allele which overcomes indica/japonica hybrid sterility (Araki *et al.,* 1988). Cui *et al.,* (1993) reported that the gene for WCV was different from that for restoring ability, which means that recombination between them forms wide compatibility restorers (WCR). Lui *et al.,* (1997) found two minor loci mapped to chromosome two and twelve. The joint effect of the two minor loci could lead to partial sterility even in the presence of S5-n. Jennings (1966) noticed close relationship between pollen and spikelet fertility observed. The correlation coefficient (r) value for pollen and spikelet fertility in all the plants of 263 hybrids studied was highly significant with the value 0.8402. Linkage relationships between wide compatibility gene (WCG), and among marker genes of C^+, esterase (Est-2) and aminopeptidase-3 (Amp3) [E.C.3.4.11.1] were detected in different recombination studies in rice. Ikehashi and Araki (1987) reported that WCG is tightly linked to C^+ (recombination frequency 3.9 to 5.6 %). Glaszmann (1987) found a tight linkage between Amp-3 and Est-2 on chromosome six (recombination frequency less than 2 %). Malic and khush (1994) found that the WCG is located 4.1 cM distant from Amp-3 and Est-2 genes.

The objectives of this study are: 1. To screen some rice germplasmes in Egypt for the presence of WCV, and 2. To study the isosyme Amp-3 as marker aided selection for WCG.

MATERIALS AND METHODS

Plant materials: This study was carried out at Rice Research and Training Center (RRTC), Sakha, Kafr El-sheikh, Egypt. Twenty-two cultivars were used including IR 36 as indica tester and Norin PL12 as japonica tester. Details of rice genotypes used are given in Table (1).

Screening for WCV: Crosses were made in greenhouse. Tester cultivars were used as male parents. Each parent was seeded in three successive dates at ten days intervals for flowering synchronization. Thirty-day-old seedlings were transplanted as single plants in the field. Female tester plants that showed initial heading were uprooted and transferred to dark room. Emasculation was done the following morning by hot water method. After pollination by the male parents, pollinated females were kept in greenhouse until harvest (Jodon 1938). Harvested panicles (25-30 days after pollination) were air-dried for 2-3 days, then hand threshed individually. The F1 grains were collected in paper envelopes and kept in oven at 50°C for five days to break dormancy.

Determination of pollen viability and spikelet fertility for declaring a variety as WCV: Pollen fertility was measured on all F1 plants. Twenty to thirty spikelets were collected from each primary panicle and fixed in 70 % alcohol. Five to six anthers were randomly taken and immerged in iodine-potassium iodide solution (1%) and examined under light microscope (40X). About 200-300 pollen grains were examined in three different fields. Unstained pollens were considered as sterile and stained ones as fertile. The pollen fertility was expressed as percentage for each F1. Spikelet fertility was expressed as percentage of the bagged panicles of all F1 plants. Varieties that gave hybrids with spikelet fertilities of at least 70 % with both indica and japonica testers were designated as WCV (Vijaya kumar and Virmani 1992).

Amp-3 isozyme studies: Amp3 isozymes were studied using starch gel electrophoresis. The plant tissues used were the plumule and coleoptile of four to six days old seedlings. Plant tissues of five seedlings were ground with fixed volumes of cold distilled water. Whatman No. 3 filter paper wicks were used to absorb the extracts. They were inserted into the gel slits to

Table (1): Genotypes and remarks of the twenty-two Egyptian and World wide collection varieties.

	Genotypes	Remarks
1	Giza 171	Japonica Egyptian rice variety
2	Giza 176	Japonica Egyptian rice variety
3	Giza 177	Japonica Egyptian rice variety
4	Giza 175	Indica Egyptian rice variety
5	Giza 178	Indica Egyptian rice variety
6	Giza 181	Indica Egyptian rice variety
7	Sakha 101	Japonica Egyptian rice variety
8	Sakha 102	Japonica Egyptian rice variety
9	IR65600-96-1-2-2	Tropical japonica
10	IR67966-44-2-3-2	Tropical japonica
11	IR68022-50-2-1	Tropical japonica
12	IR65600-188-2-2-1	Tropical japonica
13	Lemont	WCV indica line
14	Dular	WCV Australian line
15	Ballila	Japonica line
16	Morobreakan	WCV japonica line
17	IR25571-31	Indica line
18	IR50	Indica line
19	Nippon Bare	Japonica line
20	IR36	Indica line and tester
21	Norin PL 12	Japonica line and tester
22	Azucena	WCV japonica line

form a straight line parallel to the edge of the gel, to avoid distortion on sides of the papers and to facilitate comparisons of migration distances of the resulting bands. Intermixing between adjacent papers was avoided by removing excess extract with an absorbent paper prior to insertion into gel (Glaszman, 1987). A potential difference was applied through the gel, the constant parameter was the intensity, which was chosen so that the voltage would be about 10 volts/cm length of the gel. The electrophoresis run was stopped after four hours. The gel buffer used contain Trizma base (10.4 g Tris), Histidine-mono HCL (9.6g) and completed to 0.5 L distilled water, (L-leucyl-ß-Naphthylamide.HCl 1% was used as enzyme substrate). The staining buffer contains 15 mg of fast black K salt, 25 ml of Tris malate buffer 0.2M pH 3.3 and 20 ml of NaOH solution 0.1M. For staining, gels were incubated for 60 min at 40 °C.

RESULTS AND DISCUSSION

In the present study screening for wide compatibility trait was carried out. Rice F1 hybrids between indica and japonica varietal groups exhibit varying degrees of pollen and spikelet sterility (Yuan *et al.,* 1987). Several researchers have reported that certain rice varieties produce fertile F1 hybrids when crossed with indica and japonica lines (Heu 1967). Such varieties were designated as WCV providing a bridging mechanism between indica and japonica varieties in rice improvement programs (Ikehashi and Araki 1986). Results presented in table (2) showed that the percentages of pollen viability and spikelet fertility expressed as seed set in the F1s of crosses between nineteen varieties and the two testers used. In the crosses of the 19 varieties with the IR36ms indica tester percentage of pollen viability ranged from 14 to 90 %. Ten out of the 19 F1s showed more than 70 % pollen fertility. Three varieties are tropical japonica, four indica the remaining three varieties are the known WCVs Lemont, Dular and Morobreakan. Only one out of the nine varieties giving less than 70 % of pollen viability (Giza 175) was indica, the remaining varieties are japonica (tables 1 and 2).

In crosses of the 19 varieties with the Norin PL 12 japonica tester the percentage of pollen viability ranged from 25 to 90 %. Fourteen F1s showed more than 70 % pollen viability. Four varieties are tropical japonica, seven japonica and the remaining three varieties are the known WCVs (tables 1 and 2). The five varieties giving less than 70 % are all indica (tables 1 and 2). The varieties IR65600-96, IR65600-188, IR68022-50 and the three known WCVs gave more than 70 % pollen viability with both indica and japonica testers.

Spikelet fertility studies of the 19 F1s of crosses with the two testers were expressed as seed set percentage. The 19 F1s of crosses with the IR36 indica tester showed that seven out of the 19 give more than 70 % seed seting. One variety is tropical japonica, three are indica and the remaining varieties are

Table (2): The percentages of pollen viability and spikelet fertility expressed as seed set in the studied F1s progenies.

No.	Genotypes	IR 36 indica tester		Norin PL 12 japonica tester	
		Pollen viability %	Seed set %	Pollen viability %	Seed set %
1	Giza 171	14	6	85	80
2	Giza 177	48	40	82	85.4
3	Giza 176	32	38.3	85.5	91
4	Giza 175	65	60	35	30
5	Giza 178	70	60	31	25
6	Giza 181	85	89	25	15
7	IR65600-96-1-2-2	77	38	88	64
8	IR67966-44-2-3-2	55	53	77	43
9	IR65600-188-2-2-1	80	61	80	75
10	IR 68022-50-2-1	75	73	85	80.5
11	Sakha 101	41.3	48	85	96
12	Sakha 102	31.67	30	89.33	97
13	Lemont	90	85	82	92
14	Nippon Bare	40	37.5	85	90
15	IR50	80	85	35	40
16	IR25571-31	73	75	53	84.5
17	Balilla	35	25	85	80
18	Morobreakan	87	86	90	91
19	Dular	90	85	87	90
Correlation coefficient (r) =		0.9051861		0.857444221	

the three known WCVs. The 12 F1s gave less than 70 % seed set are three tropical japonica, seven japonica and two indica varieties (Giza 175 and 178). Such results are not expected for the two indica varieties. In crosses with the Norin PL 12 japonica tester 13 out of the 19 F1s showed more than 70 % seed set. Two are tropical japonica, seven are japonica, one is indica (IR25571-31) and the remaining three are the known WCVs. The six F1s which gave less than 70% seed set are two tropical japonica and four indica (tables 1 and 2). The varieties IR68022-50 and IR25571-31 in addition to the three known WCVs gave more than 70 % seed set with both indica and japonica testers. So, the IR68022-50 tropical japonica variety and the IR25571-31 indica variety are recommended to be new WCVs. Results are in agreement with previous studies. Sampath (1963) found that some indica varieties showed low compatibility with japonica varieties except Dular. Araki *et al.*, (1988) showed that the cultivar Dular (an Australian variety) showed higher compatibility with different ecotypes of japonica, Aman. Morover, Tezereh-Vijaya and Virmani (1992) screened 41 rice varieties for the WCG. The varieties Dular and Morobreakan were identified as WCVs. Malik and Khush (1994) screened 85 tropical japonica varieties from Indonesia. The varieties which had average fertility more than 70 % with both indica and japonica testers were classified as WCVs. On this basis, 21 tropical japonica varieties were classified as WCVs. Luo (1997) tested six rice varieties for WC. The results showed that three are WCV, one is partial-WCV, one is weak-WCV and one was non-WCV. Taking the classification of Luo (1997) on consideration, our results in table 2 showed that, the five varieties Lemont, Morobreakan, Dular, IR68022-50-2-1 and IR25571-31 are WCVs. The variety IR65600-188 gave from 60 to 70% seed set with the two testers could be considered as partial-WCV. The nine varieties Giza 176, IR65600-96, IR67966-44, Giza177, sakha101, sakha102, Nippon Bare, IR50 and Giza 175 which gave from 30 to 60% seed set with the two testers could be considered as weak-WCVs (table 2). The remaining five varieties could be considered as non-WCVs. The behavior of WCV classes could be explained by the findings of Lui *et al.*, (1997). They found that the major locus on chromosome six (S5) and the two minor loci that mapped to chromosome two and twelve were apparently distinct from all previously reported hybrid sterility genes. Interaction between the indica and japonica alleles at each of the loci caused a reduction in hybrid fertility. The joint effect of the 2 minor loci could lead to partial sterility even in the presence of WCG.

Correlation between pollen viability and spikelet fertility: To study the correlation between spikelet fertility expressed as seed set and pollen viability crosses with indica and japonica testers, the results were analyzed separately. An interesting observation concerning the pollen viability and

seed set was noticed. The percentages of seed set is higher than the pollen viability in 14 out of the 38 F1s studied (4 with indica and 10 with japonica tester) in table 2. This observation means that a selective mechanism in favor of viable pollens could act during pollination and before fertilization. For crosses with indica and japonica testers the correlation was positive with r = 0.9 and 0.8 respectively (table 2). Results showed that correlation is stronger in crosses with indica tester than with japonica and that pollen viability is a very important character to the pre-determination of the expected seed set, meanwhile Jennings (1966) mentioned that the pollen viability gene and WCG are different.

Results of isozyme Amp3 studies in Fig (1) showed that, eleven out of the twenty-one parental lines and varieties have the Amp3-1 activity and ten have the Amp3-2 activity. The eleven varieties that showed Amp3-1 activity were, two indica and nine japonica and all were non-WCVs. The ten varieties that showed Amp3-2 activity were, five indica, four japonica and one Australian (Dular). Six out of the ten showed to be WCVs, five in the present studies and one in previous studies (Malik and Khush, 1994). The remaining four varieties are non-WCVs. Results are in agreement with Malic and Khush (1994). They found that, the analysis of japonica varieties for allelic constitution of Amp3 gene showed Amp3-1 activity in all non-WCVs and Amp3-2 activity in all WCVs. The analysis of F2 population in crosses between the WCV Azucena and non-WCV IR36 showed a tight linkage of WCG and Amp3 gene. The WCG were found to be located onto a distance of 4.1 cM from Amp3 gene. Glaszmann (1987) reported that less than 2% recombination frequencies between Amp3 and Est 2 were found, indicating a tight linkage between the two genes. These results are in favor of the use of Amp3 gene as marker-aided in selection programs for developing WCV in rice. Although Giza 175, 178,181 and IR65600-188 are possessing Amp3-2 gene, they showed semi-sterility with the japonica tester. Results could be interpreted as follows: 1. Amp3-2 is not quiet suitable as marker for WCG in all indica varieties. 2. Indica Egyptian varieties have WCG and additional minor genes modifying hybrid fertility. Lui *et al.*, (1997) reported the presence of three loci conferring significant effect on hybrid fertility. The major locus S5 on chromosome 6 and the two minor loci on chromosomes two and twelve. The joint effect of the two minor loci could lead to partial sterility even in the presence of WCG.

In conclusion, for the Egyptian varieties and lines most of them showed less than 70 % for pollen viability and seed set with both indica and japonica testers. New international lines with WCG were found, the IR68022-50-2-1 japonica variety and the IR25571-31 indica one. In most cases a strong positive correlation between pollen viability and seed set was found. The International and newly recommended WCVs were found to possess Amp3-2

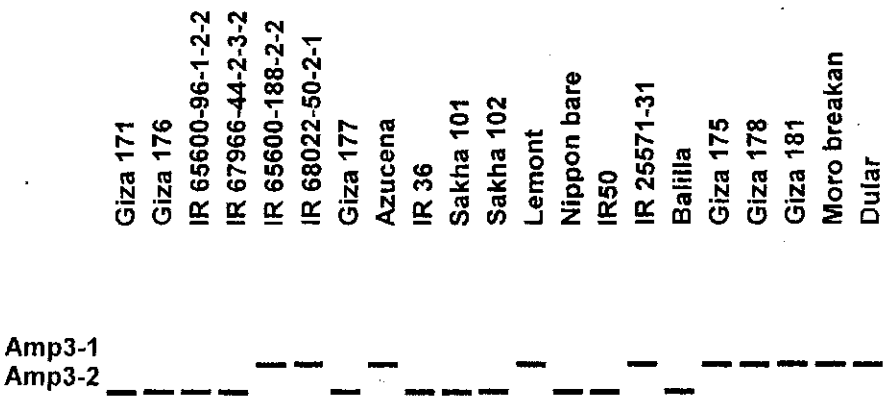

Fig. 1: The presence of Amp-3 in the evaluated varieties for WCG Ideogram shows using Amp3-2 as a marker-aided selection for wide compatible varieties.

allele, the remaining varieties which are non-WCVs possess the Amp3-1 allele. The Egyptian indica rices Giza 175, 178 and 181 even though not recommended as WCVs, since they possessed the Amp3-2 allele.

REFERENCES

Araki H.,H. Ikehashi, K. Toya and S. Matsumoto, 1988. Role of Wide Compatibility gene in hybrid rice breeding. Page79-83 "in hybrid rice", IRRI, P.O.Box933 Manila, Philippines.

Butany W.T., C. Gangadharan and M.V.S. Sastry, 1961. Present position of japonica-indica hybridization project and steps for its intensification. Proc. Rice Res. Workers conf., cuttack, 1959: 83-104. Indian council Agric. Res., New Delhi.

Cui J., Z. Yang and G.Yong, 1993. The relationship between wide compatibility and restorability in rice. Chinese J. Rice .Sci.7: 11-16.

Glaszmann J.C. (1987). Isozyme and classification of Asian rice varieties. Theo .Appl. Genetic.,74: 21-30.

Heu M.H. 1967. Studies on growth duration and hybrid sterility in remont cross breeding of cultivated rice .J. korean Society of Crop Science 4:31-71

Ikehashi H. 1982. Prospects for overcoming barriers in the utilization of indica japonica crosses in rice breeding. Oryza 19:69-77.

Ikehashi H. and H. Araki, 1984. Varietal screening for compatibility types revealed in f1 fertility of crosses in rice. Jpn. Breed. 34: 304-312.

Ikehashi H. and H. Araki, 1986. Genetics of F1 sterility in remote crosses of rice. Rice Genetics, IRRI, 11:119-130.

Ikehashi H. and H. Araki, 1987. Screening and genetic analysis of wide compatibility in F1 hybrids of distant crosses in rice (*Oriza sativa* L.) Tech.Bull.22. Tropical Agricultural Research center.

Jennings P.R. 1966. Evaluation of partial sterility in indica x japonica rice hybrids. Technical Bulletin 5, International Rice Research Institute, Los Banos, Philippines.

Jodon N.E. 1938. Experiments on artificial hybridization of rice. J. Amer. Soci. Agron., 30:294-305.

Kato S., H. Kosaka, S. Hara, Y. Maruyama and Y. Takiguchi, 1930. On the affinity of the cultivated rice plants (*Oryza sativa* L). J. Dep. Agric. Kyushu Imp. Univ. 2:241-276.

Lui K.D, J. Wang, H.B. Li, C.G. Xu, A.M. Lui, X.H. Li and Q. Zhang, 1997. A genome-wide analysis of wide compatibility in rice and the precise location of the S5 locus in the molecular map. Theo. Appl. Genet. 95: 809-814.

Luo Y.C. 1997. Identification and study on wide compatibility of six rice varieties. Journal of Anhui Agricultural Sciences, 25: 1-3.

Malik S.S and G.S Khush, 1994. Identification of wide compatibility varieties (WCVs) and tagging of WC gene with isozyme markers. Rice Genetics newsletter, 13: 121-123.

Sampath S. and H.K. Mohanty, 1954. Cytology of semi sterile hybrid. Curr. Sci., 23: 182-183.

Sampath S. 1963. The significance of hybrid sterility in rice. Proc. Symp. Rice Genetic. Cytogenetic. 1963: 175-186.

Terao H. and U. Mdusima, 1939. Some consideration on the classification of *Oryza sativa* L. into subspecies so called japonica and indica. Japan J. Bot. 10: 213-258.

Vijaya-Kumer R. and S.S. Virmani, 1992. Wide compatibility in rice (*Oriza sativa* L.) Euphytica, 64: 71-80.

Yuan I.P., S.S. Virmani and M. Xiang, 1987. Hybrid rice achievements and out look. A paper presented at the International Rice Research Conference (Sept.1987), China.

دراسات وراثية لبعض اصناف الأرز المصرية والعالمية

I- تحديد اصناف جديدة ذات مدي للقدرة التوافقية واسعا

ابراهيم العايدي[٢] ، حنفي احمد حمزة[١] ، صلاح الدين أحمد سلامه[١] ، عمرو مصطفي[٢]

١. قسم الوراثة، كلية الزراعة، جامعة المنوفية.

٢. مركز البحوث الزراعية، بسخا.

الملخص العربي

تم اجراء تلقيحات بين ١٩ صنف من اصناف الأرز المصري والعالمي مع الأصناف الأختبارية IR36 (اندبكا) و Norin PL12 (جابونيكا). قدرت النسب المئوية لحيوية لحبوب اللقاح وخصوبة السنابل في نباتات الجيل الأول لهذه التلقيحات. كما تم حساب التلازم بين نسبة عقد الحبوب وحيوية حبوب اللقاح. اظهرت نتائج الجيل الأول للتسعة عشر تلقيحا أن نسبة حيوية حبوب اللقاح تتراوح ما بين ١٤- ٩٠ % عند التلقيح مع السلالة الأختبارية انديكا حيث أن عشرة تلقيحات منها كان نسل نباتات الجيل الأول يعطي اكثر من ٧٠ % لحيوية حبوب اللقاح، بينما في التلقيحات مع السلالة الاختبارية جابونيكا تراوحت نسبة حيوية حبوب اللقاح بين ٢٥ - ٩٠ % ، حيث كان نسل الجيل الأول لاربعة عشر منها تزيد نسبة حيوية حبوب اللقاح فيها عن ٧٠ %. بالنسبة لنسبة عقد الحبوب وجد أن سبعة تلقيحات مع السلالة الأختبارية انديكا اظهرت اكثر من ٧٠ % خصوبة للسنابل وثلاث عشر تلقيحا اظهرت اكثر من ٧٠ % مع السلالة الأختبارية جابونيكا. في نباتات الجيل الأول لتلقيحات السلالات IR25571-31 & IR68022-50 & الثلاثة أصناف المعروفة مسبقا كسلالات ذات مدي للقدرة التوافقية واسعا مع كل من الأصناف الأختبارية انديكا و جابونيكا اظهرت نسبة عقد للحبوب اعلي من ٧٠ %. وبناء علية يوصي بان الصنفين IR25571-31 & IR68022-50 هي اصناف ذات مدي للقدرة التوافقية واسعا. كما انة وجد ان هناك تلازم قوي بين نسبة حيوية حبوب اللقاح ونسبة عقد الحبوب في التلقيحات مع السلالة الأختبارية انديكا يفوق ذلك المتحصل علية مع السلالة الأختبارية جابونيكا. والدراسات علي مشابهات الأنزيم Amp-3 في احدي وعشرون صنفا أن احد عشر صنفا منها تحتوي علي المشابة Amp-3-1 وعشرة أصناف تحتوى علي المشابة Amp3-2 ومن هذة العشرة الأخيرة وجد ان ستة اصناف منها ذات مدي للقدرة التوافقية الواسعه.