3rd International Conference
Sustainable Agriculture, Food and Energy

Conference Programme
Papers Abstracts

Fostering Multi-Stakeholder Collaboration on Sustainable Agriculture, Food and Energy

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SA-58

Resistance Test of West Sumatra Brown Rice Germplasm and Resistance Test to Fe Stress

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Abstract: The value of germplasm will increase if the germplasm that is equipped with the data of its resistance to abiotic stress, particularly Fe stress. The objective of this research is to study the resistance of West Sumatera brown rice germplasm to Fe stress. The experiment was conducted in farmers' fields containing 200 ppm Fe available. Materials research were exploration result of 15 genotypes of rice brown rice genotypes and two comparators of resistant genotype, Mahsuri and susceptible genotype, IR-64. Randomized block design was used in this research with 3 replications. Each rice was planted elongated among trial plots 1 line respectively. Wide of trial plot was 1.0 x 3 m^2 (3 lines with space 20 x 20 cm). The result showed that there were 4 tolerant genotypes which were Kekuningan, Siarang, Perbatasan and Talang Babungo.

Keywords : Brown rice, germplasm, Fe Stress

SA-59

Genetic Transformation Mediated Agrobacterium and Transgenic Soybean Regeneration Containing cp PSTV Genes

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Abstract: Development of plant genetic engineering technique has contributed to a new prospect in overcoming low plant production. This technique has been proved to complement a non conventional to introduce a superior gene from other organism that is isolated and constructed on a plasmid vector into plant tissue. Thus, this technique could be used to overcome the limited genetic resources in plant breeding program. We proposed the study about development of genetic transformation and transgenic soybean regeneration. The objective of this research was to develop method which transform Peanut Stripe Virus (PStV) *cp* gene mediated *Agrobacterium* into soybean . This research were conducted at Plant Molecular Biology Laboratory, and Plant Breeding Study Center Laboratory, Faculty of Agriculture, Bogor Agricultural University. Four types of Coat Protein Peanut Stipe Virus (PSTV) cp gene construction namely cp1, cp2, cp3 and cp4 were transformed into three Indonesian soybean varieties (Kerinci, Krakatau and Dempo) mediated *Agrobacterium tumafacient* strain AGLI that carrying pBNR Ti-plasmid using immature cotyledones. While three types of PStV cp gene construction namely cp2 and cp4 were transformed into MSC8606 line soybean using secondary somatic embryos (SEs) explants. The observed variables for immature cotyledone explant is (1) frquency of SEs that appear, (2) frequency of germination SEs which is kanamycine resistant (Kan^r), (3) frequency of Kan^r plantlets. While the other experiment using secondary SEs namely (1) transgenes frequency, (2) clonal amount arising from the transgene, (3) frequency of mature Kan^r SEs , and (4) frequency of Kan^r SEs which germinate. The result of this research was received 5 Kan^r plantlets which contain cp-PStV genes. All the five plantlets consissted of two Kerinci varieties, one of which contain cp1 and the other contain Cp4, still another one is Krakatau variety contain cp4, and the last two belong to Dempo variety, one contain cp2 and the other cp4. The transformation of the two cp genes (cp2 dan cp4) by using secondary SE explant was more effective than immature cotyledone. From 1965 explant which were transform for cp2 we received 113 transgenes. From 1029 secondary ES that were culture on maturing media we receive 665 mature SEs. From 665 mature SEs we receive 291 Kan^r germinated SEs.

Keywords : Agrobacterium, Transgenic Soybean, Genetic, PSTV Genes

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GENETIC TRANSFORMATION MEDIATED BY *Agrobacterium* AND TRANSGENIC SOYBEAN REGENERATION CONTAINING COAT PROTEIN GENE OF PEANUT STRIPE VIRUS

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Abstract— **The development of plant genetic engineering technique has contributed to a new prospect in overcoming low plant production. This technique has been proved to complement a non conventional one to introduce a superior gene from other organism that is isolated and constructed on a plasmid vector into plant tissue. Thus, this technique could be used to overcome the limited genetic resources in plant breeding programs. We proposed the study about development of genetic transformation and transgenic soybean regeneration. The objective of this research was to develop method which transform Peanut Stripe Virus coat protein gene mediated *Agrobacterium tumefaciens* into soybean .**

The research was conducted at Plant Molecular Biology Laboratory, and Plant Breeding Study Center Laboratory, Faculty of Agriculture, Bogor Agricultural University.Four types of coat protein gene constructions of Peanut Stripe Virus namely coat protein1, coat protein2, coat protein3 and coat protein4 were transformed into three Indonesian soybean varieties (Kerinci, Krakatau and Dempo) mediated by *Agrobacterium tumafaciens* strain *AGLI* carrying binary Ti-plasmid by using immature cotyledonary explants. While two types of coat protein gene constructions of Peanut Stripe Virus namely coat protein2 and coat protein4 were transformed into MSC8606 line soybean using secondary somatic embryos explants. The observed variables for immature cotyledonary explant is (1) frquency of somatic embryo that appear, (2) frequency of somatic embryos sprouts which are kanamycin resistant, (3) frequency of plantlets which are Kanamycin resistant. While the other experiment using secondary somatic embryos namely (1) transgene frequency, (2) clonal amount arising from the transgene, (3) frequency of mature somatic embryos that are kanamycin resistant , and (4) frequency of sprouts that are kanamycin resistant.

The result of this research received 5 plantlets which are kanamycin resistant. All the five plantlets consisted of two Kerinci varieties, one of which contains coat protein1 and the other containing coat protein4, still another one is Krakatau variety containing cp4, and the last two belong to Dempo variety, one of which contains cp2 and the other cp4. The transformation of the coat protein2 dan coat protein4 by using secondary somatic embryo explants was more effective than immature cotyledonary. From 1965 explants which were transformed for coat protein2 we received 113 transgene. From 1029 secondary somatic embryos that were culture on maturing media we receive 665 mature somatic embryos. From 665 mature somatic embryos we receive 291 sprouts that are Kanamycin resistant.

*Keywords— **Genetic transformation, Agrobacterium, transgenic, soybean, Peanut Stripe Virus***

I. INTRODUCTION

The application of genetic engineering technique in obtaining plant resistant to attack of plant diseases is an alternative promising, because the resistance found in wild type species is difficult to assemble conventionally into commercial plants.This is because the nature of sexual incompatibility.

The effort to assemble transgenic plants having superior character will be continuously treated by remembering that genetic engineering technique appears as a breakthrough against things that cannot be done by using the conventional breeding, especially in obtaining plant resistant to diseases.

Genetic transformation technique for introduction of certain genes into the plant tissues with the assistance of *Agrobacterium* is more easily applied because this technique is relatively easy to be applied and does not need a sophisticated equipment .Although it has been reported effective for some plants, but the application on soybean require further development .

At present there are three forms of resistance against an attack of the virus obtained through genetic transformation. All the three forms of resistance are by using satellite RNA sequence, antisens RNA sequence, and coat protein (cp) gene. Cp gene is the best if compared to satellites RNA and antesens RNA. This gene has broad spectrum because besides causing resistance to the virus concerned, they also cause resistant to the same group of viruses [6]; [7]. The resistance spectrum of transgenic plant that carries cp gene against other viruses correlated with the degree of the similar amino acids that are expressed with amino acid

sequences of viruses cp which infect (Beachy, 1990).

Agrobacterium as a tool transformation gene into the plant tissue really needs certain environment so that we can obtain transformed cells as many as possible .The purpose of this research is to get transgenic soybean that contain PStV cp gene.

I. MATERIALS AND METHOD

Preparation of *Agrobacterium* : *Agrobacterium tumefaciens* strain AGLI that carry binary Ti-plasmid were obtained from Qweensland Agricultural Biotechnology Center. This plasmid contains neophosphotransferase II (NPTII) as marker gene and peanut stripe virus coat protein (PStV cp) gene. Coat protein (cp) gene consisting of four constructions namely cp1(PStV cp consist of promotor, open reading frame and terminator will express PStV cp, cp2 (PStV cp consist of promotor, open reading frame and terminator undergo point mutation will express modification of PStV cp), cp3(PStV cp consist of promotor and terminator that undergo modification frame shift will express mRNA but does not form cp, and cp4 (PStV cp consist of promotor, open reading frame and terminator undergo deletion will express shorter PStV cp). *Agrobacterium* grown in Luria Broth (LB) media with the addition of rimfapicin (20 mg / media litre) and kanamycin (50 mg / media litre). Media pH is set up on 7.0.

Agrobacterium from glycerol stock are grown in liquid LB media for 24 hours by shaking at 100 rpm .To get a single colony, bacteria grown in solid LB media for two days and then the single colony of bacteria is grown in liquid LB for 24 hours. Bacteria are harvested by centrifugation at 7000 rpm and 7^0C temperature for 10 minutes. Pellets that are obtained from the results of centrifugation are washed with liquid Murashige and Skoog (MS) media , then once again they are centrifugated. Furthermore we do re-suspension again in the liquid MS so we obtain bacteria suspension .The concentration of bacteria used is 0.5 x 10^7 on optical density 600 nm $(OD)_{600}$ by spectrophotometer.

Explant preparation : Immature cotyledonary explants from three Indonesian soybean varieties namely kerinci , krakatau and dempo and one soybean line namely MSC8606 are isolated from immature pods are harvested from main plants. Before cotyledons are isolated , the pods are first sterilized by immersion in chlorox 20 % , after that they are shaken for 20 minutes and then rinsed with sterile water. Cotyledonary are isolated by using sterile scalpel blades and pincers .While explants secondary somatic embryos are obtained from the results of recycle primary somatic embryos that is induced by Naphthalene Acetic Acid (NAA) (10 mgs / l media) + 2-4D 10 mgs / l media .

Pre-culture : Pre culture was done for source of explants from immature cotyledonary for 10 days .Pre culture conditions prevent the explants from dying due to *Agrobacterium* infection on inoculation and co-cultivation steps. In addition pre culture will make explants active growing toward the formation of embryogenic calluse that allows to become more competent to transformation.

Preparation of Selection Media : Media for Somatic embryos (SEs) induction that were prepared at pH 5.8 put into stock bottles measuring one litre .Gelatinous (8 grms / litre media) put on media while it was shaken and then it was sterilized with autoclave on pressure 17.5 *psi* for 30 minutes . Sterile media was cooled with water-bath until temperature 50^0C and than as carried into *laminar air flow* where 100 mgs/litre kanamycin was added and then the mixture was poured into culture bottles of about 20 mls/bottle.

Inoculation and co-cultivation : Explants that have been pre cultured for 10 days were inoculated with *Agrobacterium* in the concentration 0.5 x 10^7 cells / ml media at OD_{600} for 10 minutes. Inoculation was conducted by immersing explants into bacterial suspension. Then the explants were taken with pincers one by one to be placed on sterile paper tissue and then explants were co-cultivated in culture media for one week. Furthermore the explants were re-cultured into SE induction media with the addition of cefotaxime (300 mgs/l media) for five days. This experiment conducted transformation of four types of PStV cp gene constructions mediated by *Agrobacterium* into soybean namely cp1, cp2, c3,p and cp4.

Maintenance : The explants were re cultured into the SE induction media that were added antibiotics kanamycin (100 mgs / 1) and cefotaxime (300 mgs / 1). Kanamycin is usefull for reducing the growth of normal cells that can develop into somatic embryos (SEs). In this case those which are not tranformed will die out or sensitive kanamycin (Kan^s), while transgenic cells remain alive and develop into selection media or resistant kanamycin

(Kan^r). Cefotaxime antibiotic function to kill *Agrobacterium* isolates. The formation of SEs on selection media indicate that transformation has taken place inside the soybean. for clarifying this indication SEs that grow were germinated into germination media remain to possess kanamycin. Furthermore sprouts were re cultured in the same media once in two weeks until plantlets are formed

SEs maturation : Every SEs that survive on selection media were re cultured in selection media without plant growth regulator (PGR) for one month. During this period SEs will develop and enlarge. In order to SEs free from PGR on this media charcoal was added with a dosage of 2 mgs/l media.

SEs germination : SEs were re cultured on germination media that contain Gibberellic Acid 3 (GA3) with a dosage of 3 mgs/l media for 15 days. Then re-cultured on MS media contain Benzyl Amino Purine (BAP).

Observation : Observations on this experiment was conducted when the explants were two months old in the selection media. In this case cells or explants that are not transform died indicated by the change of color are become brown and dry. The variable that can be observed are as follows: (1) The frequency of SEs appearences that Kan^r (2) The frequency of SEs sprouts Kan^r (3) The frequency of forming plantlets Kan^r (4) Transgene frequency (5) The number of clonals that appear from transgene.

III. RESULTS AND DISCUSSION

The result of the observation shows the appearance of SEs and sprouts Kan^r that can be seen in Table 1. This table shows that all the four constructions of PStV cp gene are transform into three varieties of Indonesian soybean obtain SEs Kan^r for three cp gene namely cp1, cp2 and cp4. Table 1 also shows that 17 sprouts of SEs Kan^r are obtained consisting of 6 sprouts from kerinci variety (two sprouts are indicated contain cp1 gene, one sprout contain cp2 and three sprouts contain cp4 gene), three sprouts are from krakatau variety (one sprout contain cp2 gene and two sprouts contain cp4 gene). For Dempo variety we obtain 8 sprouts Kan^r each consists of 3 sprouts containing cp1 gene, 2 sprouts containing cp2 gene and 3 sprouts containing cp4 gene.

The sprouts that we obtain are re cultured in regeneration media containing kanamycin (selection media) once in two weeks so that we obtain plantlets Kan^r containing PStV cp gene. Until the end of this experiment 5 plantlets are obtained (the representation can be seen on picture 1.c). all the five plantlets consist of 2 plantlets belong to kerinci variety (1 plantlet contain cp1 gene and the other one contain cp gene), 1 plantlet belong to krakatau variety, and 2 plantlets belong to dempo variety (1 plantlets contain cp2 and the other one contain cp4 gene (Table 1)

The transformation of two cp gene constructions (cp1 and cp4) on secondary SE explants are found more effective than immature cotyledonary explants. The result of the observation show that from 1965 explants which are transformed cp2 we obtain 113 transgene or about 5.81% (see Table 2). In table 2 we can also see that cp4 which were transformed into 1080 explants we obtain 63 transgene.

After it was re cultured for 5 weeks in SE induction media containing kanamycin appear secondary SEs and after it was re cultured in media free PGR we obtain mature SEs. From 1029 secondary SEs which have been cultured on maturing media we obtain 665 mature SEs (Table 3). In table 3 we can also see that from 665 mature SEs we obtain 291 sprouts Kan^r (Figure 1.b).

As a whole the frequency SEs Kan^r gained from this experiment we achieved the result is significantly high, in this case we found 333 SEs from 12.000 explants experimented but they are difficult to be regenerated to become plantlets. From this experiment we have found only 5plantlets, all the five plantlets are not successfully aclimated because the fenomenon of its growth in the field is different from control plants. Up to the present we don't know yet with certainty the factor that prevents their regeneration.

The experiment using socondary SEs explants is promise to obtain transgenic soybean that contain PStV cp gene because this method result to gain a greater amount of SEs and sprouts Kan^r whose frequency is higher when compared with immature cotyledonary. The high frequency transgene can be understood because explants that have been transformed are secondary SEs due to the fact that each SE consists one cell so that if they are transformed they will grow and develop in selection media and form a new SEs.

TABLE 1.

THE FREQUENCY OF SEs, SPROUTS, AND PLANTLETS KAN^R WHICH ARE TRANSFORMED WITH FOUR PStV CP GENE CONSTRUCTIONS ON THREE SOYBEAN VARIETIES.

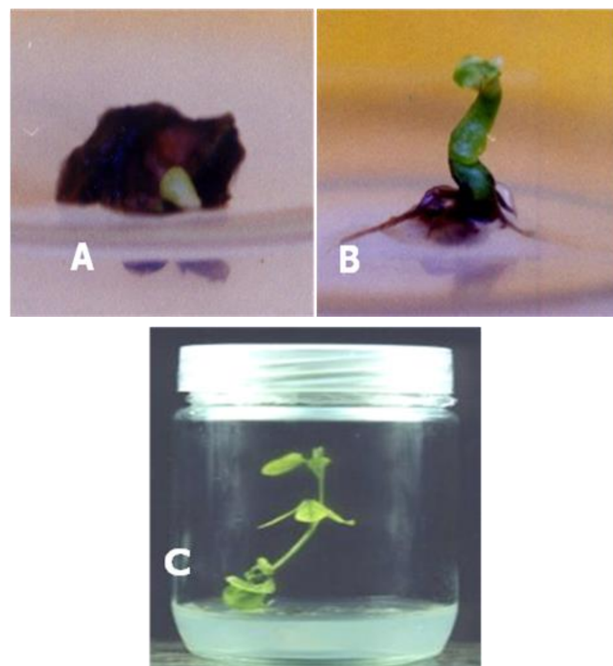
Varieties pstv cp	Amount of Explants	Amount of SEs	Amount of Sprouts	Amount of plantlets
Kerinci :				
CP 1	1000	34	2	1
CP 2	1000	27	1	-
CP 3	1000	0	0	0
CP 4	1000	30	3	1
Karakatau :				
CP 1	1000	24	0	0
CP 2	1000	29	1	0
CP 3	1000	0	0	0
CP 4	1000	32	2	1
Dempo :				
CP 1	1000	52	3	0
CP 2	1000	48	2	1
CP 3	1000	0	0	0
CP 4	1000	57	3	1
Total	**12.000**	**333**	**17**	**5**

TABLE 2 .
THE FREQUENCY AND TRANSGENE PERCENTAGE WHOSE SUBSTANCE A MADE FROM SECONDARY SEs EXPLANTS MSC8606 SOYBEAN LINE THAT HAVE BEEN TRANSFORMED WITH PStV CP GENE .

No. exp.	PStVcp types	Amount of Explants	Amount of Transgene	Pct.
1	CP2	480	23	4.8
2	CP2	555	35	6.3
3	CP2	600	33	5.5
4	CP2	330	22	6.7
5	CP4	450	29	6.4
6	CP4	630	37	5.9
Total		3045	179	

TABLE 3 .
THE FREQUENCY OF SEs SECONDARY , MATURE SEs , AND SPROUTS KAN^R WHOSE SUBSTANCE ARISE FROM SECONDARY SEs EXPLANTS MSC8606 SOYBEAN LINE THAT HAVE BEEN TRANSFORMED WITH PStV CP GENE.

No. exp	PStV cp	Amount of Transgene	Amount of Clonal	Amount of Mature SEs	Amount of Elongation SEs
1	CP2	23	122	94	30
2	CP2	35	217	158	63
3	CP2	33	188	118	46
4	CP2	22	145	79	42
5	CP4	29	168	95	47
6	CP4	37	189	121	63
Total		179	1029	665	219



Picture. Representation of somatic embryo, sprout and plantlet soybean that are kanamycin resistant. (A) somatic embryo, (B) sprout, and (C) plantlet.

IV. CONCLUSION

1 . Three out of four PStV cp gene constructions namely cp1, cp2 and cp4 can be transformed into soybean. Proved by obtaining SEs, sprouts and plantlets Kan^r.
2. The explants from secondary SEs easier to be transformed. We obtain more SEs and sprouts when compared to immature cotyledonary explants.

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