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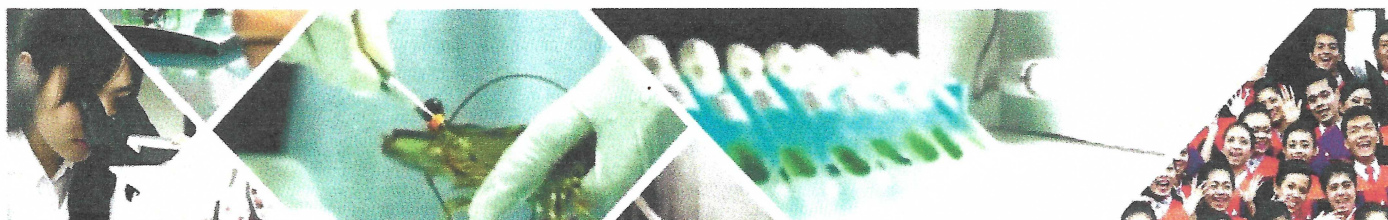
CERTIFICATE OF PRESENTER

This is to certify that

Reny Herawati

**has participated in the 2nd International Symposium on
Sustainable Agriculture and Agro-Industry (ISSAA 2017),
Walailak University, THAILAND
held on 28th-29th March 2017**

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Acting on behalf of the President
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The 2nd International Symposium on Sustainable Agriculture and Agro-Industry (ISSAA)

ISSAA 2017: Innovation for tomorrow's world

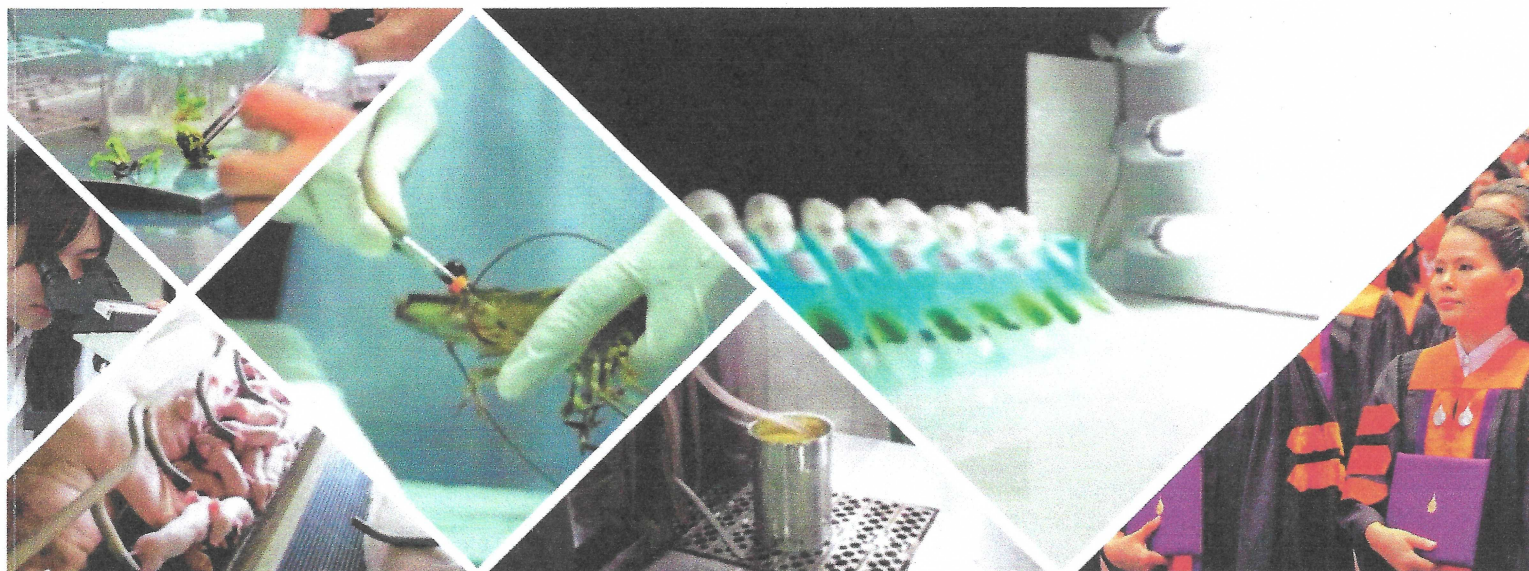
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Screening of double haploid new plant type upland rice for drought stress using polyethyleneglycol (PEG) and proline analysis

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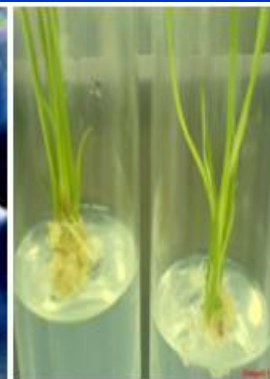
ABSTRACT

The selection process of lines, especially to drought stress can be done by knowing the characteristics of physiology and biology as well as the morphology of the root system of each genotype. A number of doubled haploid (DH) lines were developed through anther culture from the cross of a new plant type (Fatmawati) and upland rice varieties. The purpose of this research was to identify doubled haploid lines tolerant to drought stress, as well as to determine the consistency of the polyethyleneglycol (PEG) 6000 test at germination stage and drought stress in the greenhouse. Materials used in this study were 78 double haploids (DH2) lines and 4 parents (SGJT-28, SGJT-36, Way Rarem, and Fatmawati), Jatiluhur and Cisokan as tolerance and sensitive control. The method used in this experiment were the early selection of doubled haploid (DH2) with 20% polyethyleneglycol (PEG) 6000 at germination stage and proline analysis at the vegetative stage. The results showed that PEG 6000 inhibited germination (33.9 percent), root length (60.8 percent) and shoot length (80 percent) of upland rice lines. Drought stress treatment (60 percent of field capacity) at the flowering period showed no significant reduction in the growth of doubled haploid upland rice but reduced the weight of grains per hill (52.11 percent). Drought stress decreased in total chlorophyll ($20.7 \mu\text{mol cm}^{-1}$) and increased proline content in leaves ($30.3 \mu\text{mol g}^{-1}$). Lines tested with polyethyleneglycol (PEG) 6000 were not all consistent with simulated drought stress in the greenhouse. Field experiment should be done to get accurate results.

Keywords: Drought stress, doubled haploid, upland rice, polyethyleneglycol, proline

SCREENING OF DOUBLE HAPLOID NEW PLANT TYPE UPLAND RICE FOR DROUGHT STRESS USING POLYETHYLENEGLYCOL (PEG) AND PROLINE ANALYSIS

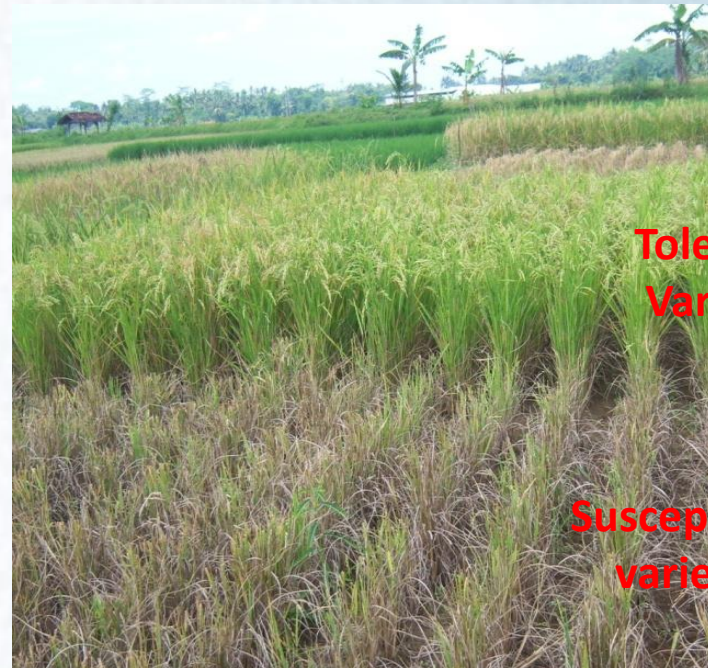
By:
Reny Herawati
Bambang S Purwoko
Iswari S Dewi



Problems in Upland Rice

- 1. Low Productivity
- 2. Drought

- Rate National Productivity for upland rice less than 4/ha
- Rate National Productivity for rice reach 6/ha

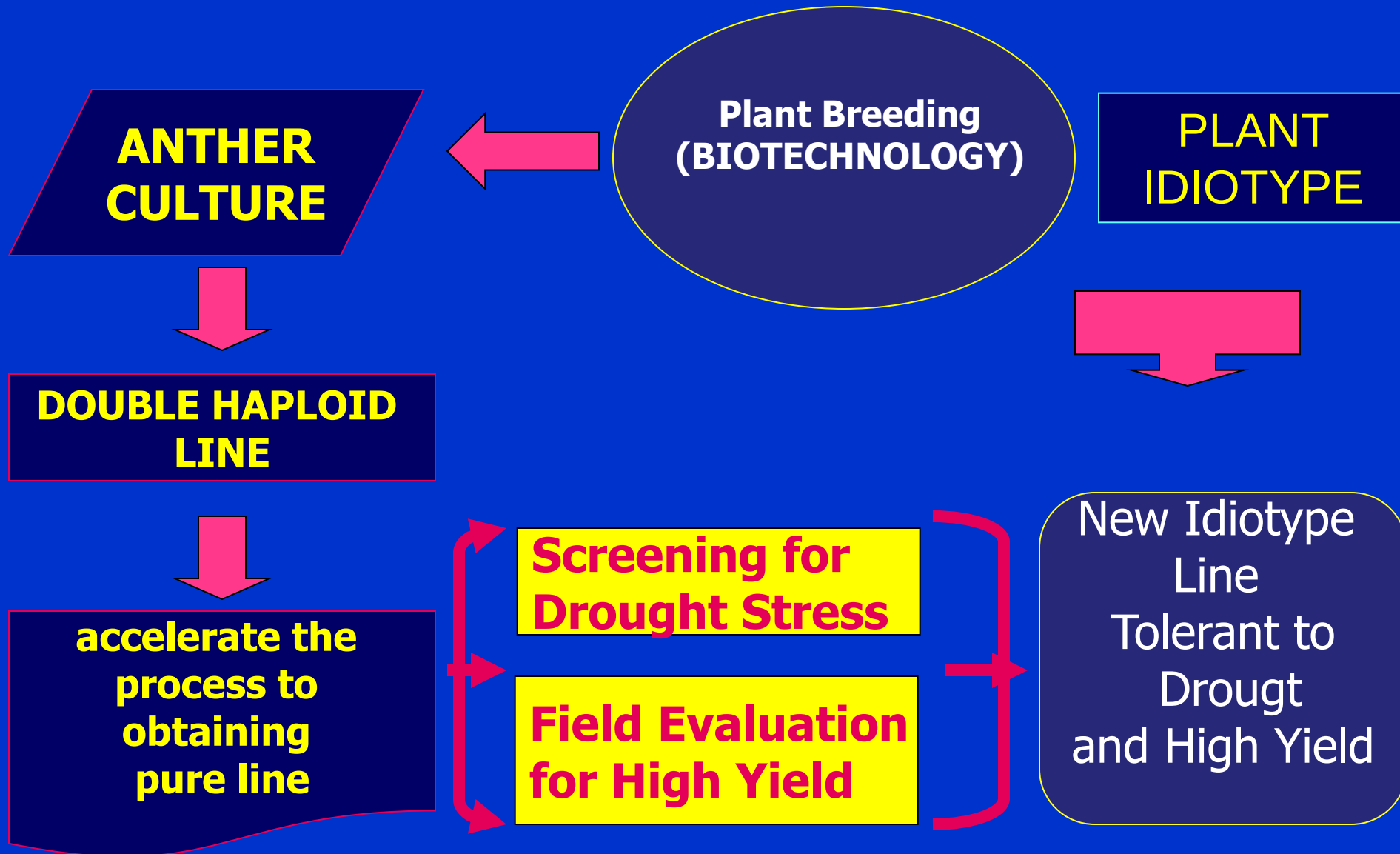


New Genotype

Drought Stress

High Yielding and Tolerant to Drought

LOW INPUT APPROACH



A X B

F1

F2

F3

F8 (Stable Lines)

evaluation

CONVENTIONAL BREEDING

A X B

F1

Anther Culture

(spontaneous double haploid)
DH0

Dihaploid homozygote

DH1 (Stable Lines)

evaluation

ANTHER CULTURE TECHNIQUE

THE COMPARISON BETWEEN CONVENTIONAL BREEDING AND ANTHER CULTURE
TECHNIQUE

The Aims of the Research

To develop a new plant type of upland rice through anther culture and to evaluate the tolerance of doubled haploids (DH) lines to drought stress



MATERIALS:

F1 CROSSES

Fatmawati X Way Rarem(P1)

Fatmawati X SGJT-28 (P2)

Fatmawati X SGJT-36 (P3)

FIR:

Way Rarem X Fatmawati (P4)

SGJT-28 X Fatmawati (P5)

SGJT-36 X Fatmawati (P6)

Anther Culture

- F1 Plant
- Media N6
- Media MS



Media for Callus induction
(N6) : 2.0 mg/l NAA + 0.5
mg/l kinetin + 10^{-3} M

Regenerasi media(MS) : 0.5
mg/l NAA dan 2.0 mg/l
Kinetin + 10^{-3} Putresin

Rooting Media(MS) : 0.5
mg/l IBA dan 40 g/l
maltosa + 10^{-3} M putresin

ANTHER INOCULATION in N6 MEDIUM



a. Pre treatment in 5°C, 8-10 days



b. Selection of panicles



c. Eksplant Sterilize



d. Anther Inoculation in N6 medium

Callus Induction

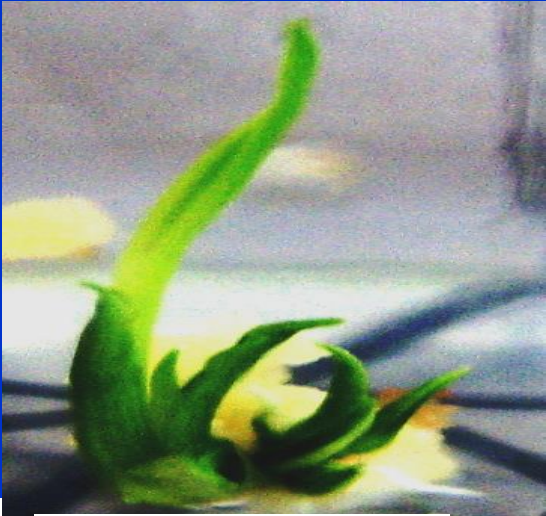
Callus Induction, $t\ 25\pm2^{\circ}\text{C}$, in dark room



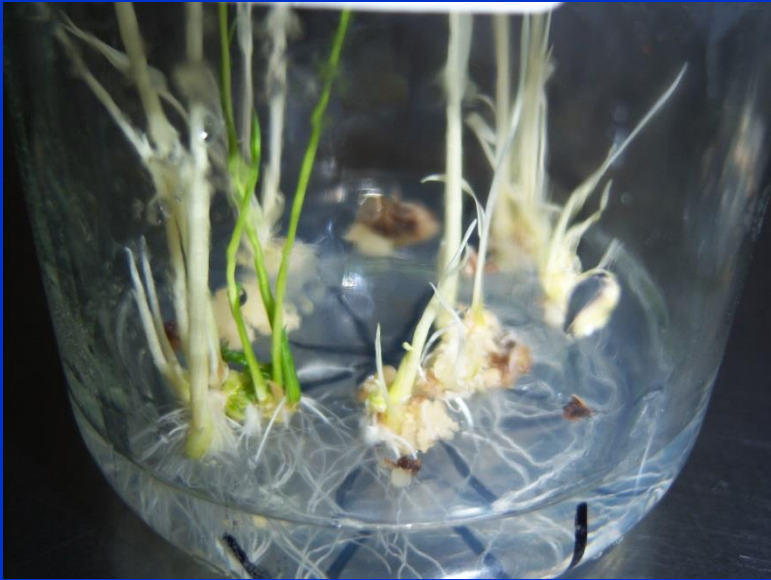
a. Calli mikrospore



b. Callus in N6 Medium



c. Green Plant

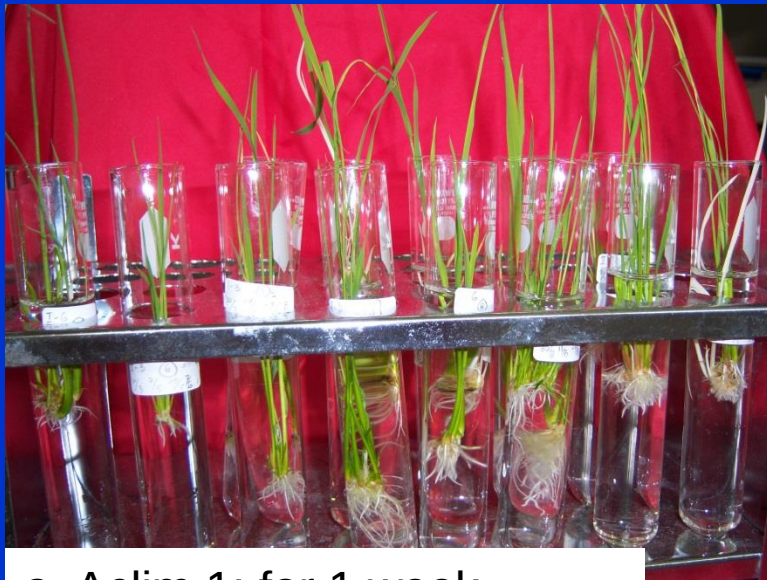


d. Green Plant and Albino (3-5 cm)



d. Green Plant in rooting medium

ACCLIMATISATION



a. Aclim 1: for 1 week



b. Aklim 2: 1 week



c. Planted in pot



d. Selected haploid/DH

Doubled Haploid Lines were Obtained through Anther Culture from New Type Rice Fatmawati Crosses

Crosses	Potensial Plantlet	Aclimatisation Plant	Planted Plant	Doubled Haploid
P1(Fatmawati x Way Rarem)	22	13 (59.1)	13 (100)	6 (46.2)
P2 (Fatmawati x SGJT-28)	24	22 (91.7)	22 (100)	13 (59.1)
P3 (Fatmawati x SGJT-36)	428	387 (90.4)	364 (94.1)	187 (51.4)
P4 (Way Raremx Fatmawati)	3	3 (100)	3 (100)	3 (100)
P5 (SGJT-28 x Fatmawati)	9	9 (100)	7 (77.8)	5 (71.4)
P6 (SGJT-36 x Fatmawati)	291	252 (86.6)	242 (96.0)	134 (55.4)
Total	777	686 (88.3)	651 (94.9)	348 (53.5)

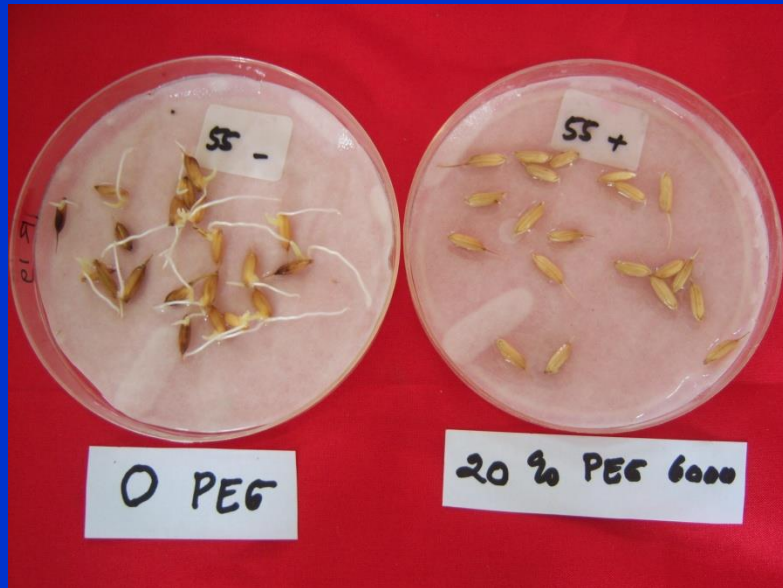
We obtained doubled haploid lines more than 53.5% from total green plant which it's regenerated .

Screening of Doubled Haploid Lines through Anther Culture for Drought Stress

Objective :

to identify Doubled Haploid Lines through Anther Culture for Blast Drought Stress

EVALUASI GALUR HAPLOID GANDA HASIL PERSILANGAN PADI GOGO DENGAN PADI TIPE BARU TERHADAP CEKAMAN KEKERINGAN



20 % PEG 6000
Observation after 24 jam
during 6 days:
-% germination
-Root length
-plumule length

-100 % FC dan 60 % FC at
flowering stage
-masing2 4 galur dalam
kriteria PAR pada Uji PEG:
Tenggang,
Moderat, dan
Peka

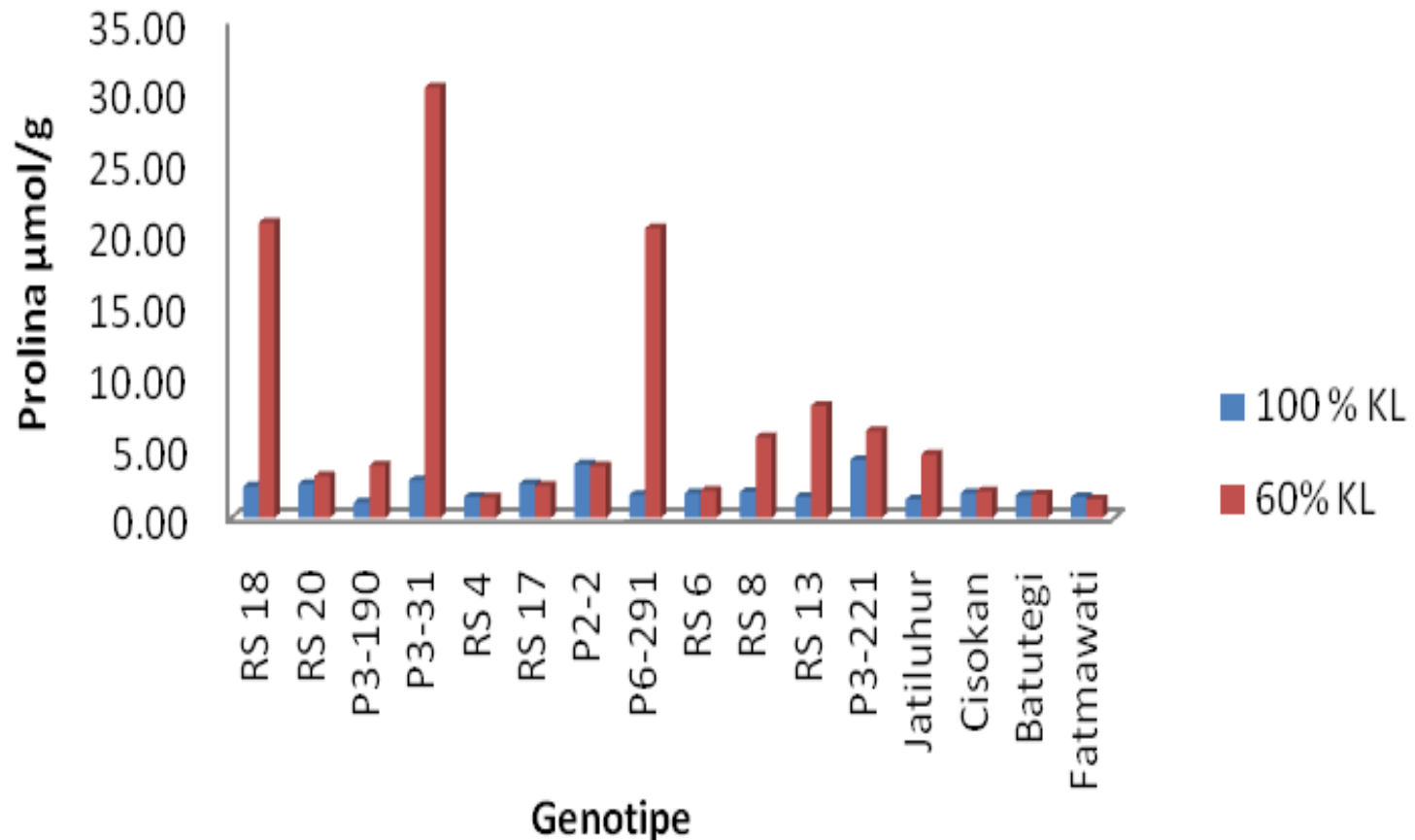
Hasil seleksi galur haploid ganda padi gogo hasil kultur antera berdasarkan panjang akar relatif (PAR) pada 20 % PEG 6000					
Persilangan	Jumlah Galur				
	Toleran	Agak Toleran	Moderat	Agak peka	Peka
P1 (Ftmwati x W Rarem)	0	1	0	0	0
P2 (Ftmwati x SGJT-28)	1	3	2	0	0
P3 (Ftmwati x SGJT-36)	7	3	14	3	7
P4 (W Rarem x Ftmwati)	0	1	1	0	0
P5 (SGJT-28 x Ftmwati)	0	1	0	0	0
P6 (SGJT-36 x Ftmwati	2	4	12	5	11
Jumlah	10	13	29	8	18

Bobot gabah per rumpun pada kondisi normal dan tercekam kekeringan dan Nisbah Bobot gabah per rumpun galur haploid ganda hasil kultur antera

GALUR	Uji PEG	Bobot gabah/rumpun (g)			Uji cekaman kekeringan
		100 % KL	60 % KL	NBGR	
RS 18	Toleran	4.31	0.28	6.49	Peka
RS 20	Toleran	14.87	3.31	22.26	Peka
P3-190	Toleran	28.44	19.94	70.11	Toleran
P3-31	Toleran	17.74	3.88	21.87	Peka
RS 4	Moderat	19.16	18.22	95.09	Toleran
RS 17	Moderat	10.40	8.18	78.65	Toleran
P2-2	Moderat	17.00	3.09	18.17	Peka
P6-291	Moderat	8.07	2.20	27.26	Peka
RS 6	Peka	16.98	12.20	71.85	Toleran
RS 8	Peka	3.32	0.83	25.00	Peka
RS 13	Peka	24.61	2.62	10.65	Peka
P3-221	Peka	8.66	1.32	15.24	Peka
Jatiluhur	Toleran	52.34	33.01	63.07**)	Toleran
Cisokan	Peka	38.03	11.75	30.89	Peka
Batutegi	Toleran	46.77	31.37	67.07	Toleran
Fatmawati	Peka	38.89	15.55	39.98	Peka

-Pengujian PEG tidak semua konsisten dengan pengujian cekaman kekeringan
 -Disarankan untuk menggunakan metode uji PEG pd stadia bibit umur 7 hari

Kandungan prolina pada cekaman kekeringan galur haploid ganda padi gogo hasil kultur antera



Evaluasi Karakter Agronomi Galur Haploid Ganda Padi Gogo Tipe Baru Hasil Kultur Antera di Lahan Kering

BAHAN DAN METODE :

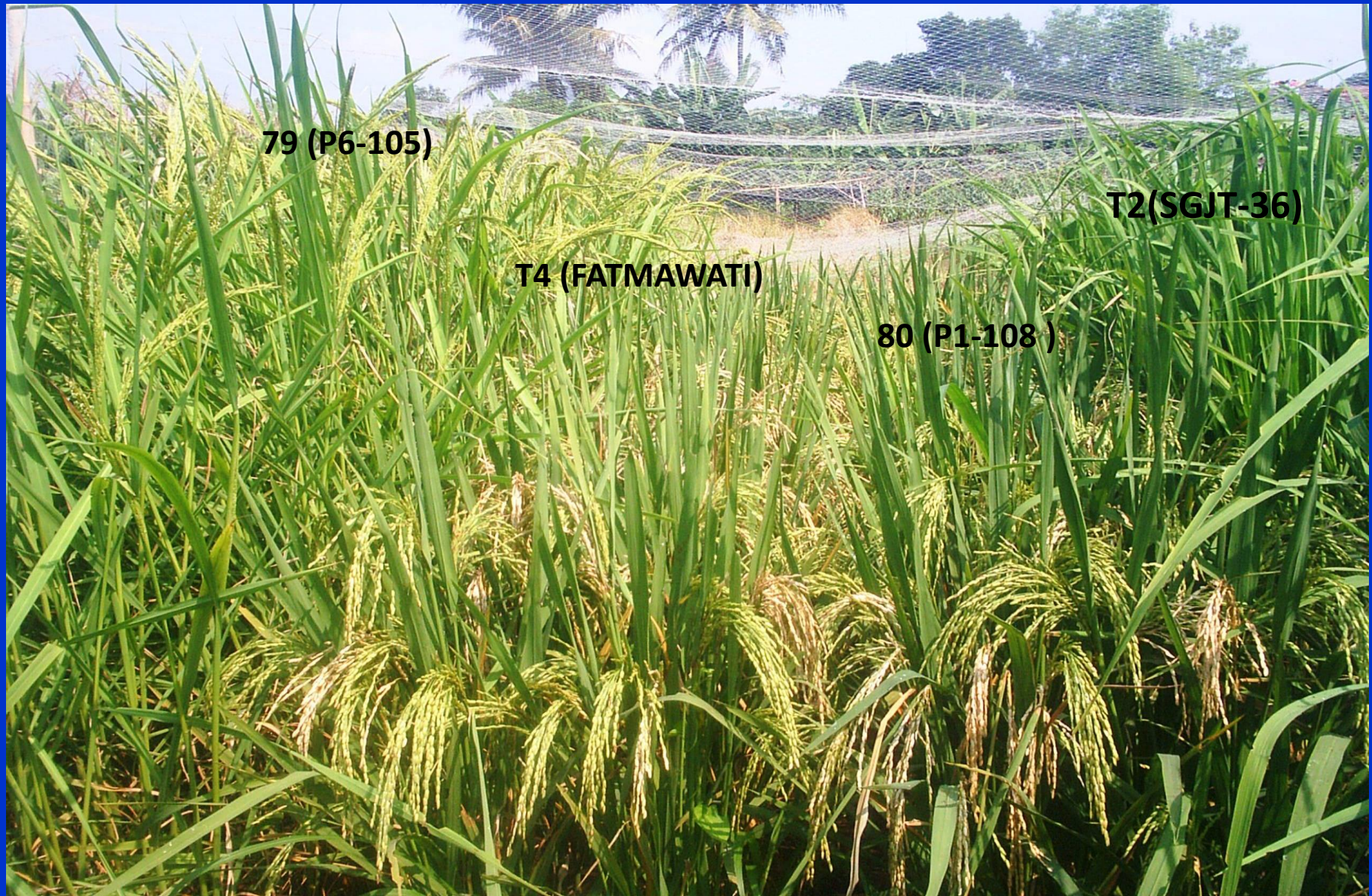
- ❖ 20 galur haploid ganda
- ❖ 4 cek : Fatmawati,
Way Rarem,
SGJT-36,
SGJT-28
- ❖ Petak berukuran 4 m x 4 m
dua baris tiap galur
- ❖ Jarak tanam 20 cm x 30 cm
- ❖ Rancangan Augmented
(Baihaki 2000).



Pengamatan :

Tinggi tanaman, umur panen,
jumlah anakan produktif,
jumlah gabah, dan
bobot gabah/rumpun.

EVALUASI 20 GALUR HAPLOID GANDA PADI GOGO TIPE BARU HASIL KULTUR ANTERA DI LAHAN KERING



CONCLUSION

- ❖ Anther culture could obtain pure line of rice and resistant to blast disease.
- ❖ Thorough anther culture was obtained 348 doubled haploid lines and prepare to evaluated.
- ❖ Screening for blast disease was obtained 24 doubled haploid lines resistant to all ras (ras 173, 033, dan 001), 1 doubled haploid line resistant to ras 033 dan 173, 16 doubled haploid lines resistant to ras 033 dan 001, 3 doubled haploid lines resistant to ras 033, dan 9 doubled haploid lines resistant to ras 001.
- ❖ The selected lines should be further evaluated to determine the stability of the characters in each generation, especially for testing the results in the field.

A photograph of a lush green rice field with golden-brown rice stalks in the foreground. The text "Thank you" is written in large, bold, 3D letters with a yellow-to-orange gradient, slanted diagonally across the center of the image. The background shows a line of trees under a bright sky.

Thank you

Galur P3-27



Tinggi Tanaman : 163.5

Anakan produktif : 9.5

Umur berbunga 83

Umur Panen : 115

Panjang malai : 30.45

Gabah isi : 201.16

Gabah hampa : 177.87

Bobot 1000 butir : 27.82

Bobot gabah/rumpun : 31.88

Potensi Hasil : 5.31 ton/ha

Tahan Blas (Ras 173, 033, 001)

Agak toleran Al

Galur P3-28



Tinggi Tanaman : 162.17
Anakan produktif : 97.17
Umur berbunga : 81.15
Umur Panen : 115
Panjang malai : 32.06
Gabah isi : 178.56
% Gabah hampa : 42.14
Bobot 1000 butir : 27.82
Bobot gabah/rumpun : 30.02
Potensi Hasil : 5.00 ton/ha
Tahan Blas (Ras 173, 033, 001)
Agak toleran Al

Galur P5-50



Tinggi Tanaman : 129 cm

Anakan produktif : 14

Umur berbunga : 77 hari

Umur Panen : 110 hari

Panjang malai : 26.96 cm

Gabah isi : 162.42

Gabah hampa : 31.72

Bobot 1000 butir : 20.58 g

Bobot gabah/rumpun : 37.92 g

Potensi Hasil : 6.32 ton/ha

Tahan Blas (Ras 001)

Agak toleran Al

Galur P6-105



Tinggi Tanaman : 155.21 cm

Anakan produktif : 12

Umur berbunga : 84 hari

Umur Panen : 111 hari

Panjang malai : 29.19 cm

Gabah isi : 211.2

Gabah hampa : 176.62

Bobot 1000 butir : 24.59 g

Bobot gabah/rumpun : 41.53 g

Potensi Hasil : 6.92 ton/ha

Tahan Blas (Ras 033)

Toleran Al

Galur P3-162



Tinggi Tanaman : 162.17 cm
Anakan produktif : 9.64
Umur berbunga : hari
Umur Panen : 111 hari
Panjang malai : 30.53 cm
Gabah isi : 242.52
Gabah hampa : 188.81
Bobot 1000 butir : 22.95 g
Bobot gabah/rumpun : 41.44 g
Potensi Hasil : 6.91 ton/ha
Tahan Blas (Ras 173, 033, 001)
Toleran Al

Galur P3-204



Tinggi Tanaman : 151.5 cm

Jumlah anakan : 9.83

Anakan produktif : 9.5

Umur berbunga : 79 hari

Umur Panen : 101 hari

Panjang malai : 30.58 cm

Gabah isi : 160.56

Gabah hampa : 147.39

Bobot 1000 butir : 28.36 g

Bobot gabah/rumpun : 31.03 g

Potensi Hasil : 5.17 ton/ha

Tahan Blas (ras 033 dan 001)

Sangat toleran Al