Improvement of Inpari 30 and Situ Bagendit rice varieties for tolerance to drought through spike-stalk injection method

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Abstract. Extreme climate change requires rice varieties adaptable to drought condition. Adaptation will play an important role in ensuring the sustainability of food security. This research aimed to improve drought tolerance of Inpari 30 and Situ Bagendit varieties through Spike-Stalk Injection Method (SIM). DNAs from several plant species, such as rice (cv. Cabacu), grasses (*Echinochloa crusgalli/E. colona*, elephant grass/*Pennisetum purpureum, Bothriochloa pertusa*, *Cenchrus echinatus*, *Sorghum nitidum*, *Ischamemum timorensis* and *Guinea grass*), sugarcane, wild rice (*Oryza nivara*), maize and sorghum were injected to tillers of cultivar Inpari 30 and Situ Bagendit. The first set of M_1 Situ Bagendit-SIM and M_1 Inpari 30-SIM seeds were treated with 20% of PEG 8000 solution for 10 days. Some of the well germinated seeds were planted in pots and maintained untill harvest. The selected M_2 Situ Bagendit-SIM and M_2 Inpari 30-SIM and the second set of M_1 Situ Bagendit-SIM and M_1 Inpari 30-SIM seeds were planted at Muara Experimental Station, Bogor (West Java). The result of PEG 8000 assay showed that M_1 Situ Bagendit-SIM-elephant grass, sugarcane and *O. nivara* had significantly longer radicle length and higher fresh weight compared to Situ Bagendit, while M_1 Inpari 30-SIM-sugarcane, sorghum and elephant grass had significantly longer radicle and plumule length, and higher radicle weight compared to Inpari 30. Field trial showed that the mutant lines of Situ Bagendit-SIM performed better than those of Inpari 30-SIM. The grain weight of M_1 Situ Bagendit-SIM-Cabacu, *jajagoan* grass, maize, sugarcane, *O. nivara* and *B. pertusa*, and M_2 Situ Bagendit-SIM-*O. nivara* and elephant grass were higher than that of Situ Bagendit. The grain weight of M_1 Inpari 30-SIM-*jajagoan* grass was also higher than that of Inpari 30. Therefore, SIM could be an alternative way to develop genetic variation of rice plant.

Keywords: DNA injection, field trial, grasses, mutation, PEG 8000.

1. Introduction

Drought affects rice plants in various aspects. Plant responses during drought stress at the cellular and molecular levels include cell volume reduction, decreasing leaf area, increasing root-to-shoot ratio and

stomatal sensitivity [1]. These responses are also accompanied by a reduction in photosynthesis rate; an increase in the accumulation of dissolved osmotic compounds, such as proline, betaine sugar and alcohol sugar; changes in enzyme activity and gene expression; and production of long and thick roots and formation of cuticle layers in leaves [2].

The lack of water in the root area causes slow cell division activity in the root meristem area, facilitating to reduce the dry weight of the root besides decreasing the potential of water [3].Therefore, drought-tolerant rice varieties are expected to overcome this problem and able to produce grains, even though under water limitation.

Spike-Stalk Injection Methods (SIM) is one of methods for developing new rice lines done by Chinese National Hybrid Rice Research researchers [4], which was inspired by the injection method into rye [5]. This method has been successfully applied on rice by injecting grass DNA (*Echinochloa crusgalli*) to rice stems when the plants are in early booting stage [4]. DNA is expected to be carried through the water and nutrients flow (through xylem) into the rice genome during meiosis. SIM can overcome the barriers of inter-specific hybridization of cultivated rice and non-AA genome wild rice during chromosomes pairing. This method has been used to generate new rice germplasm by injecting DNAs from a variety of plant species such as wild rice, maize, sorghum, *E. crusgalli* and *Pannicum maximum* [4], and resulted in several hybrid rice varieties [6].

Inpari 30, also known as Ciherang-*Sub1* rice variety, is an irrigated rice with submergence tolerant [7]. It is necessary to combine submergence tolerant properties with drought tolerance in rainfed rice field. Situ Bagendit is an upland rice which was released in 2003. This variety is well adapted in irrigated and upland rice field. The performance of Inpari 30 and Situ Bagendit rice varieties is preferred by farmers [8]. This study aimed to improve Inpari 30 and Situ Bagendit rice varieties by increasing their drought tolerance through SIM.

2. Materials and methods

This research was conducted in a glasshouse of Indonesian Center for Agricultural Biotechnology and Genetic Resources Reserch and Development (ICABIOGRAD) and in a field at Muara Experimental Station (250 m asl), Bogor, from January 2016 to July 2017. Two rice varieties, Inpari 30 and Situ Bagendit, were grown in pots in the glasshouse until panicle formation. Genomic DNAs from several plant sources (Table 1) were isolated following the method of Doyle and Doyle [9]. DNA concentration was adjusted to 450 ng/µl in 1× SSC solution. A total of 50 µl of DNA solution was injected gradually to the second node from the bottom of the panicle following the method of Zhao et al. [4]. Injected panicles were maintained until seed maturity.

2.1. PEG 8000 drought tolerance phenotypic selection

The first set of M_1 seeds were derived from Inpari 30 and Situ Bagendit which were injected with sorghum, elephant grass, wild rice (*O. nivara*), *(E. crusgalli*/*E. colona)*, sugarcane and Cabacu rice (hereafter named as M_1 Inpari 30-SIM or M_1 Situ Bagendit followed by the name of DNA source). Seeds of these mutants were surface sterilized with 70% ethanol solution for 5 minutes. The seeds were then washed three times with sterilized distilled water. Germination assays were performed by evenly distributing the seeds in 10-cm-diameter sterilized petri dishes. Each dish was moistened with 10 ml distilled water and germinated for 3 days.

Germinating seeds of similar size or those with 2 mm of plumule and radicle length were selected. The seeds were transferred to 10 cm-diameter sterilized Petri dishes and immersed for 10 days in 20 ml distilled water containing 20% of polyethylene glycol (PEG) 8000 solution [10], which gave osmotic potential of -5,11 bar or -0,511 Mpa [11]. The experiment was designed in a completely randomized design (CRD) with three replications. The length of plumule and radicle, as well as fresh weight, was measured from 10 germinating mutant seeds of each line selected randomly. The M_1 seedlings from the PEG treatment were planted to potted soil and maintained until the M_2 Inpari 30 and M_2 Situ Bagendit seeds set.

Table 1. List of plant species used as DNA injection sources.

Source	Species/cultivar/common name/accession number	Origin
Rice	*Oryza sativa* cv. Cabacu	Brazil
Wild rice	*O. nivara* 103840 (Reg. 05012-00001)	Philippines (ICABIOGRAD collection)
Maize	Lamuru (Reg. 05002-03696)	Maros District, South Sulawesi Province (ICABIOGRAD collection)
Grass	*Jajagoan* grass/*Echinochloa crusgalli* (= *E. colona*)	Bogor, West Java Province
Grass	Elephant grass/*Pennisetum purpureum*	Bogor, West Java Province
Sugarcane	-	Bogor, West Java Province
Sorghum	Mutiara Kulonprogo L70 (Reg. 05005-00074)	Kulon Progo District, Yogyakarta Province (ICABIOGRAD collection)
Grass	*Bothricochloa pertusa*	Kupang, East Nusa Tenggara Province
Grass	*Cenchrus echinatus*	Kupang, East Nusa Tenggara Province
Grass	*Sorghum nitidum*	Kupang, East Nusa Tenggara Province
Grass	*Ischamemum timorensis*	Kupang, East Nusa Tenggara Province
Grass	Guinea Grass	Kupang, East Nusa Tenggara Province
Grass	Molato Grass	Kupang, East Nusa Tenggara Province

2.2. Field test

M_1 and M_2 seeds from both varieties were sown in 3 m × 6 m plots at Muara Experimental Station, Bogor (250 m asl), without replication. These populations consisting of 13 lines of M_1 Inpari 30-SIM, 13 lines of M_1 Situ Bagendit-SIM, 7 lines of M_2 Inpari 30-SIM (sown in 35 plots) and 7 lines of M_2 Situ Bagendit-SIM (sown in 35 plots). Both wild types (Inpari 30 and Situ Bagendit), IR20 (drought-sensitive check variety) and Cabacu (drought-tolerant check variety) were also sown. The agronomic characters of these mutants were compared to those of the wild types.

3. Results and discussion

3.1. PEG 8000 screening

The initial screening for drought-tolerance of M_1 Inpari 30-SIM and M_1 Situ Bagendit-SIM used 20% of PEG 8000 solution (w/v). After 10 days of incubation in the solution, longer radicle and/or plumule and higher radicle fresh weight than those on wild types were generally observed on SIM mutant lines (Table 2 and Table 3, Figure 1).

The average percentage of germinated seeds from PEG 8000 selection of M_1 Inpari 30-SIM was 15.07% with the highest rate was shown by M_1 Inpari 30-SIM-*O. nivara* (24.1%) and the lowest was by M_1 Inpari 30-SIM-elephant grass (8.6%). The average percentage of germinated seeds from PEG selection of M_1 Situ Bagendit-SIM was 17.37% with the highest rate was in M_1 Situ Bagendit-SIM-sorghum (41.1%) and the lowest was in M_1 Situ Bagendit-SIM-*O. nivara* (7,3%) (data not shown).

Radicle length of M_1 Inpari 30-SIM and M_1 Situ Bagendit-SIM showed significant difference compared to its wild types (Inpari 30 and Situ Bagendit). Radicle length of M_1 Inpari 30-SIM-sugarcane, -sorghum, -elephant grass, -Cabacu and -*E. crusgalli* were longer than that of Inpari 30 (Table 2). Similarly, radicle length of M_1 Situ Bagendit-SIM-elephant grass, -sugarcane and -*O. nivara* were longer than that of its wild type (Table 3).

The plumule length of M_1 Inpari 30-SIM-sugarcane and M_1 Inpari 30-SIM-*E. crusgalli* significantly differed and exceeded that of the other M_1 Inpari 30-SIM and its wild types (Table 2). On

the contrary, the plumule length of M_1 Situ Bagendit-SIM and the wild type were not significantly different, except for M_1 Situ Bagendit-SIM-*O. nivara* (Table 3).

The fresh weight of germinated seeds of M_1 Inpari 30-SIM and M_1 Situ Bagendit-SIM showed significant difference compared to their wild types. The fresh weight of M_1 Inpari 30-SIM-sorghum, -SIM-sugarcane, -*E. crusgalli*, -Cabacu, and -*O. nivara* were higher than that of Inpari 30 (Table 2). Likewise, the fresh weight of M_1 Situ Bagendit-*O. nivara*, -elephant grass, -sugarcane, -Cabacu, -*E. crusgalli* and -maize were higher than those of its wild types (Table 3).

Table 2. Plumule and radicle length of M_1 Inpari 30-SIM and M_1 Situ Bagendit-SIM mutants after treatment with 20% of PEG 8000 solution for 10 days.

No.	Rice variety/mutant	Plumule length (cm)[a]	Radicule length (cm)	Germinated seed fresh weight (g)
1	Cabacu	0.94±0.12 ab	4.71±0.61 b	0.041±0.01 ab
2	Inpari 30	1.18±0.64 bc	3.16±0.92 a	0.031±0.01 d
3	Inpari 30-SIM-*E. crusgalli*	1.87±1.03 a	3.41±0.98 cd	0.040±0.01 ab
4	Inpari 30-SIM-maize	1.09±0.32 bc	0.33±0.30 f	0.031±0.00 d
5	Inpari 30-SIM-elephant grass	1.38±0.52 b	3.67±0.68 c	0.035±0.01 cd
6	Inpari 30-SIM-sorghum	1.32±0.58 bc	4.59±0.70 b	0.043±0.01 a
7	Inpari 30-SIM-sugarcane	2.18±0.71 a	6.04±0.80 b	0.042±0.01 ab
8	Inpari 30-SIM-Cabacu	1.21±0.50 bc	3.42±0.85 cd	0.039±0.01 abc
9	Inpari 30-SIM-*O. nivara*	1.20±0.41 bc	1.55±0.37 e	0.037±0.01 bc

[a] Values followed by the same letter within one column are not significantly different at 5% level according to DMRT.

Table 3. Plumule and radicle length of M_1 Situ Bagendit-SIM and M_1 Situ Bagendit-SIM mutants after treatment with 20% of PEG 8000 solution for 10 days.

No.	Rice variety/mutant	Plumule length (cm)[a]	Radicle length (cm)	Germinated seed fresh weight (g)
1	Cabacu	1.45±0.12 b	3.16±0.61 b	0.042±0.007 a
2	Situ Bagendit	0.84±0.22 b	0.32±0.20 d	0.031±0.009 d
3	Situ Bagendit-SIM-*E. crusgalli*	0.88±0.33 b	1.22±0.53 c	0.036±0.006 bc
4	Situ Bagendit-SIM-maize	0.75±0.15 b	0.97±0.66 c	0.035±0.006 bc
5	Situ Bagendit-SIM-elephant grass	0.86±0.32 b	4.62±0.77 a	0.039±0.004 ab
6	Situ Bagendit-SIM-sorghum	0.95±0.36 b	3.27±0.76 b	0.033±0.007 cd
7	Situ Bagendit-SIM-sugarcane	0.83±0.31 b	4.14±0.76 a	0.037±0.006 bc
8	Situ Bagendit-SIM-Cabacu	0.93±0.30 b	1.03±0.65 c	0.036±0.005 bc
9	Situ Bagendit-SIM-*O. nivara*	1.45±0.74 a	4.51±1.45 a	0.042±0.007 a

[a] Values followed by the same letter within one column are not significantly different at 5% level according to DMRT.

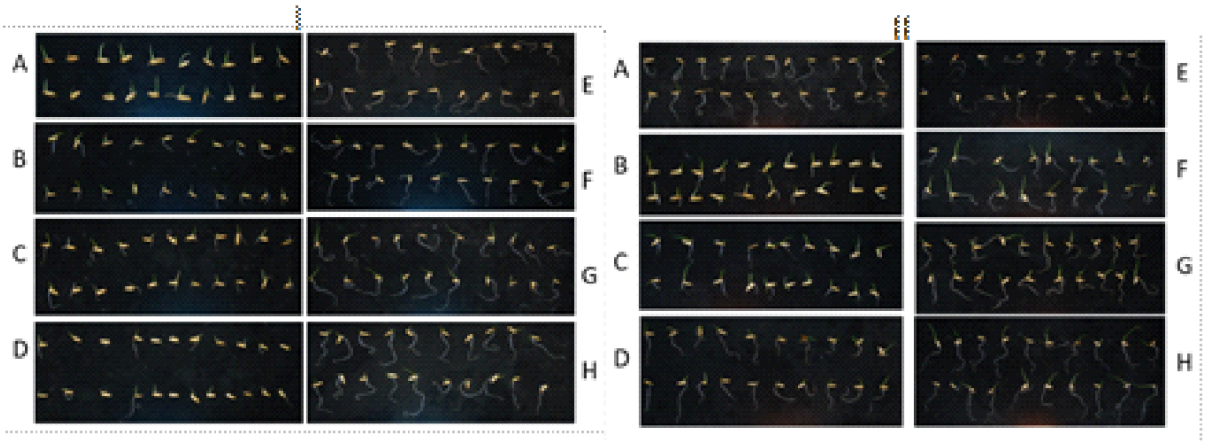

Figure 1. The performance of radicle and plumule of M_1 Inpari 30-Spike-Stalk Injection Method (SIM) mutants and M_1 Situ Bagendit-SIM mutants after treated with 20% of PEG 8000 solution for 10 days.
I. A = Situ Bagendit (wild type), B–H = mutants derived from Situ Bagendit injected with DNA from rice cv. Cabacu, *jajagoan* grass (*E. crusgalli*), maize, sugarcane, sorghum, *O. nivara* and elephant grass, respectively.
II. A = Inpari 30 wild type, B–H = mutants derived from Inpari 30 injected with DNA from maize, *O. nivara*, rice cv. Cabacu, sorghum, *jajagoan* grass, elephant grass and sugarcane, respectively.

3.2. Field test

The selected mutant lines from the first set (M_2 Inpari 30-SIM and M_2 Situ Bagendit-SIM) and the second set (M_1 Inpari 30-SIM and M_1 Situ Bagendit) were planted in the field experimental station. The results of the field test can be seen in Table 4 and 5.

M_1 and M_2 Inpari 30-SIM mutant lines were significantly different from the wild type for all parameters, except for the panicle length. These mutant populations showed shorter plant height and also lower number of filled grain, 100-grain weight and the total grain weight. However, the panicle number of M_1 Inpari 30-SIM-*E. crusgalli*, -SIM-*B. pertusa*, and -SIM-molato grass; and M_2 Inpari 30-SIM-sugarcane, and -SIM-maize mutants were higher than that of Inpari 30. On the contrary, M_1 Inpari 30-SIM-*E. crusgalli* had higher grain weight than the wild type (Table 4).

The agronomic performance of M_1 and M_2 Situ Bagendit-SIM mutant lines were not significantly different to that of the wild type (Table 5). Nevertheless, the number of panicle in M_1 Situ Bagendit-sugarcane and M_2 Situ Bagendit-*O.nivara* were slightly higher than that of Situ Bagendit (Table 5). Additonally, the grain weight of M_1 Situ Bagendit-SIM-Cabacu, -SIM-*E. crusgalli*, -SIM-maize, -SIM-sugarcane, -SIM-*O. nivara*, and -SIM-*B. pertusa* and M_2 Situ Bagendit-SIM-*O. nivara* and -SIM-elephant grass exceeded that of its wild (Table 5).

Results from the PEG treatment and the field test were consistent. In PEG assay, DNA from *E. crusgalli*, sugarcane, maize and *O. nivara* resulted in longer plumule and radicle and higher fresh weight of germinated seeds, which corresponded to longer panicle length and higher grain weight in the field (Table 2–5).

Table 4. Agronomic profile of M_1 and M_2 Inpari 30-SIM mutants grown in Muara Experimental Station, Bogor in 2017.

Rice variety/mutant[a]	Plant height (cm)[b]	Number of panicles	Panicle length (cm)	Number of grains/ panicles	Number of empty grains/ panicles	100-grain weight (g)	Grain weight/ plant (g)
IR20	93.35b-d	14.86de	25.02b	122.70a	33.69d-h	2.80a	18.99c-h
Cabacu	111.31a	10.41e	24.83b	58.38e-j	45.48c-h	2.90a	8.34e-h
Situ Bagendit	89.98c-h	20.33a-d	24.71b	116.93ab	15.05h	2.43ab	37.41a
Inpari 30	93.94bc	16.25c-e	24.55b	100.64a-c	30.90d-h	2.49ab	22.51b-e
M_1 Inpa-SIM-sorghum	89.08c-i	19.60a-d	23.30b	90.00b-e	23.08f-h	2.50ab	32.56a-c
M_1 Inpa-SIM-Cabacu	82.30ij	21.10a-d	21.66b	60.00e-i	44.60c-h	2.29a-d	6.45e-h
M_1 Inpa-SIM-*E. crusgalli*	94.93bc	26.00a	25.33b	96.27a-d	34.27d-h	2.40ab	41.12a
M_1 Inpa-SIM-maize	87.73c-i	17.00b-e	23.86b	83.67c-e	44.07c-h	2.24a-d	12.20e-h
M_1 Inpa-SIM-sugarcane	88.40c-i	20.90a-d	23.14b	80.20c-f	42.15c-h	2.11a-d	22.17b-e
M_1 Inpa-SIM-*O. nivara*	84.80g-j	18.60a-d	23.10b	70.20c-g	37.20d-h	2.36a-c	19.43c-h
M_1 Inpa-SIM-elephant grass	90.60d-g	20.60a-d	22.90b	37.80h-j	62.60c-e	2.33a-c	5.37f-h
M_1 Inpa-SIM-*B. pertusa*	90.60d-g	25.20ab	24.64b	99.60a-c	25.60e-h	2.44ab	36.69ab
M_1 Inpa-SIM-*C. echinatus*	83.00h-j	20.20a-d	24.20b	95.00a-d	27.20e-h	2.34a-c	21.45c-f
M_1 Inpa-SIM-*S. nitidum*	85.87e-j	23.73a-c	23.24b	64.33d-h	31.07d-h	2.43ab	16.46d-h
M_1 Inpa-SIM-*I. timorensis*	80.20j	23.12a-d	22.36b	83.92c-e	21.28gh	2.27a-d	19.06c-h
M_1 Inpa-SIM-Molato grass	86.25d-j	25.75a	22.80b	31.38ij	75.00bc	2.47ab	5.15gh
M_1 Inpa-SIM-Guinea grass	90.80d-g	22.40a-d	26.76b	88.60b-e	46.80c-h	2.55ab	20.63c-g
M_2 Inpa-SIM-sorghum	98.08b	21.04a-d	25.14b	64.48d-h	43.68c-h	2.39ab	14.99d-h
M_2 Inpa-SIM-Cabacu	92.72b-e	20.72a-d	24.11b	45.28g-j	46.48c-h	2.26a-d	10.86e-h
M_2 Inpa-SIM-*E. crusgalli*	85.10f-j	20.90a-d	23.17b	28.60j	60.30c-f	1.09e	3.46h
M_2 Inpa-SIM-maize	92.33d-f	25.33ab	32.80ab	29.87ij	97.33ab	1.45c-e	8.44e-h
M_2 Inpa-SIM-sugarcane	89.04c-i	30.04a-d	23.90b	49.68f-j	98.88ab	1.41de	6.90e-h
M_2 Inpa-SIM-*O. nivara*	85.60e-j	15.35c-e	27.60ab	83.80c-e	112.40a	1.76c-e	12.62e-h
M_2 Inpa-SIM-elephant grass	84.80f-j	18.00a-e	24.45b	65.48d-h	59.04c-g	2.42ab	10.70e-h
CV (%)	5.095	25.64	28.44	26.49	50.05	2.65	52.88

[a] Inpa = Inpari 30.

[b] Values followed by the same letter within one column are not significantly different at 5% level according to DMRT.

Seed germination and seedling development are very important for early establishment of plants under stress condition. It has been reported that seed germination and early seedling growth are potentially the most critical stages for water stress [12] and drought stress impairs the seed germination [13]. Therefore, analysis of germination and seedling growth traits and their response to drought can be useful for selection of rice genotypes tolerant to drought [14].

PEG solution is commonly used in drought tolerance stress experiments because its ethylene content binds water, making it inaccessible to roots and resulting in plant dryness [15]. Either PEG 8000 solution at 20% concentration [10] or PEG 6000 solution at 25% concentration [16] can be used for drought tolerance selection. In this study, we used 20% of PEG 8000 solution as drought determinator to mimic drought stress condition. In the previous study, seed germination was severely affected by 20% of PEG solution [17]. Under water stress, low water potential is a determining factor for inhibiting seed germination [18].

Table 5. Agronomic profile of M_1 and M_2 Situ Bagendit-SIM mutants grown in Muara Experimental Station, Bogor in 2017.

Rice variety/mutant[a]	Plant height (cm)[b]	Number of panicles	Panicle length (cm)	Number of grains/ panicles	Number of empty grains/ panicles	100 grain-weight (g)	Grain weight/plant(g)
IR20	93.35 b-d	14.86 h	25.02 a-f	122.7 a-e	33.69 ab	2.80 ab	18.99 g
Cabacu	111.31 a	10.41 i	24.83 a-g	58.38 g	45.48 a	2.90 a	8.34 h
Inpari30	93.94 b-d	16.25 d-h	24.55 b-g	100.64 ef	30.90 a-d	2.40 a-c	22.51 fg
Situ Bagendit	89.99 b-e	20.33 a-e	24.71 a-g	116.93 a-e	15.05 de	2.43 a-c	37.40 a-e
M_1 Situ-sorghum	93.75 b-d	15.25 gh	25.13 a-f	120.60 a-e	23.15 b-e	2.45 a-c	34.90 a-f
M_1 Situ-Cabacu	100.15 b	17.10 c-h	26.05 a-c	144.55 a	14.75 de	2.56 a-c	46.82 a
M_1 Situ-*Echinocloa crusgalli*	98.60 bc	19.35 a-h	25.50 a-d	137.85 ab	16.20 c-e	2.52 a-c	38.64 a-e
M_1 Situ-maize	95.25 b-e	17.30 c-h	26.53 a	135.15 a-c	15.55 c-e	2.38 a-c	41.04 a-d
M_1 Situ-sugarcane	91.50 b-e	22.30 a	26.50 a	115.45 a-e	16.50 c-e	2.57 a-c	42.31 a-c
M_1 Situ-*Oryza nivara*	87.20 c-e	20.00 a-f	25.00 a-f	115.20 a-e	8.80 e	2.35 a-c	38.37 a-e
M_1 Situ-elephant grass	91.52 b-e	20.96 a-c	24.93 a-g	97.92 ef	45.60 a	2.38 a-c	35.41 a-e
M_1 Situ-*Bothricochloa pertusa*	93.95 b-d	20.85 a-d	25.61 a-d	137.75 ab	31.65 a-c	2.06 c	44.56 ab
M_1 Situ-*Cenchrus echinatus*	87.00 c-e	15.85 e-h	23.04 g	101.10 ef	24.05 b-e	2.14 bc	27.15 e-g
M_1 Situ-*Sorghum nitidum*	88.75 b-c	16.70 c-h	25.08 a-f	101.35 ef	22.70 b-e	2.42 a-c	29.30 d-f
M_1 Situ-*Ischamemum timorensis*	89.47 b-e	15.53 f-h	25.19 a-e	106.93 ef	11.50 e	2.69 a-c	28.36 d-f
M_1 Situ-Molato grass	92.47 b-d	20.07 a-f	24.54 b-g	115.40 a-e	12.80 e	2.31 a-c	38.61 a-e
M_1 Situ-Guinea grass	87.30 c-e	18.57 a-h	24.91 a-g	116.83 a-e	19.87 b-e	2.50 a-c	31.21 c-f
M_2 Situ-sorghum	93.29 b-d	17.77 a-h	25.10 a-f	104.45 d-f	24.50 b-e	2.37 a-c	29.01 d-f
M_2 Situ-Cabacu	86.45 de	17.53 b-h	24.28 c-g	94.98 ef	17.45 b-e	2.54 a-c	32.32 b-f
M_2 Situ-*E. crusgalli*	86.82 c-e	16.87 c-h	23.24 e-g	118.35 a-e	16.80 c-e	2.54 a-c	34.36 a-f
M_2 Situ-maize	82.93 d-f	15.51 f-h	23.71 d-g	98.14 ef	25.05 b-e	2.47 a-c	30.91 d-f
M_2 Situ-sugarcane	79.73 ef	19.55 a-g	23.18 fg	83.51 f	19.90 b-e	2.49 a-c	32.90 b-f
M_2 Situ-*O. nivara*	87.15 c-e	22.02 ab	24.79 a-g	110.12 b-f	18.33 b-e	2.57 a-c	42.91 a-c
M_2 Situ-elephant grass	83.21 d-f	20.18 a-e	24.48 c-g	100.22 e-f	21.47 b-e	2.54 a-c	44.99 ab
CV (%)	9.05	16.87	5.16	17.6	49.05	18.82	24.79

[a] Situ = Situ Bagendit.

[b] Values followed by the same letter within one column are not significantly different at 5% level according to DMRT.

Our results showed that M_1 Inpari 30-SIM-*E. crusgalli* and -sugarcane and M_1 Situ Bagendit-SIM-*O. nivara* and -sugarcane, showed longer radicle and plumule, and also higher fresh weight. These characteristics can help plants to tolerate drought conditions. Long and large root volume will enable plants to adapt in the field with less water. Henry et al. [19] and Comas et al. [20] mentioned that root

architecture, such as diameter, specific root length,and root density, are some important parameters for selecting rice plants for drought tolerance.

The application of SIM has changed the agronomic character in Inpari 30, either for the better or the worse, whereas positive influence of SIM were observed for Situ Bagendit. Zhao et al. [21] mentioned two premises of DNA fragments integrated to rice genome that (1) the expression of gene products in the target rice derived from the inserted DNA fragments retain the intact structures including gene upstream and encoding regions and (2) the regulatory factors of the transcription machinery in rice recognize the upstream sequences of the inserted genes.

In this study, the injected DNA from *E. crusgalli* resulted in higher grain weight in M_1 Inpari 30 and M_1 Situ Bagendit and higher number of panicles in M_1 Inpari 30, but it did not result in significant change in Inpari 30 for other parameters. Rice is a typical C3 plant, whereas *E. crusgalli* is a C4 plant. This weed exhibits fast growth with higher stems, wider and darker green leaves, high rate of photosynthesis, and high efficiency in water and mineral use [22]. DNA fragments transferred from *E. crusgalli* to rice are responsible for exerting the unknown influence on the expression of rice genes [21].

The injected DNA from maize resulted in higher number of panicles in M_1 Inpari 30 and higher number of grain weight in M_1 Situ Bagendit. Li et al. [23] mentioned that the transfer of exogenous corn DNA may induce mutations in rice. If the mutations occur in the functional regions of the genome, this may influence expression of this gene, leading to changes in expression, structure and function of proteins while phenotype variation may also occur in later generations. Ji et al. [24] reported that exogenous corn DNA could change the expression of proteolytic enzymes in mutant rice seedlings, possibly as a result of variation of bases.

The injection of DNA from sugarcane resulted in higher number of panicle and higher grain weight in M_1 Situ Bagendit and higher number of panicles in M_2 Inpari 30. A similar effect was observed for the injection of DNA from wild rice *O. nivara*, where higher grain weight in M_1 and M_2 Situ Bagendit and higher panicle number panicle in M_2 Situ Bagendit were obtained. Zhao et al. [6] reported the development of Yewei B rice from a mutant of V20B through injection DNA of wild rice *O. minuta.* The molecular analysis revealed high DNA polymorphism exists between the mutant and its receptor, indicating that the special DNA fragment from *O. minuta* may be integrated into the genome of the mutant.

The integration of foreign DNA into Inpari 30 and Situ Bagendit genome, respectively, can be proven molecularly. Several class of molecular markers, such as the transferability of rice SSR to Poacea family [25], AFLP [23], as well as SNP markers can be used to detect base differences between wild type and SIM mutants [26,27].

The SIM method has also been analyzed by DNA sequencing [28], SSR, AFLP, RAPD and RFLP markers [6,23,28], differential proteomes [21], whole genome sequencing [29] and restriction-site associated DNA sequencing technology (RADseq) [30].

Results between the PEG treatment corresponded well with the results of the field test for the lines derived from the injection of DNA from *E. crusgalli*, sugarcane, maize and *O. nivara*. This consistency is in agreement with the hypothesis that specific DNA segments might integrate into the genome of the cultivated rice and could be stably passed onto the offsprings [31]. Therefore, SIM can be an effective approach to transform genomic DNA of distantly related species for creating new rice germplasm.

4. Conclusions

Spike-Stalk Injection Method (SIM) on tillers of Inpari 30 and Situ Bagendit rice varieties with DNAs of various plant species have resulted in M_1 mutant seedlings with longer radicle and plumule length and higher fresh weight compared to their wild types. M_1 and M_2 Situ Bagendit-mutants had better agronomic performance than Inpari 30-mutants. Therefore, SIM could be an alternative way to develop genetic variation of rice plant.

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