Marker traits association of agronomical traits correlated with stagnant flooding tolerance in rice

T Sitaresmi\textsuperscript{1,2,*}, D W Utami\textsuperscript{3}, W B Suwarno\textsuperscript{4}, S W Ardie\textsuperscript{4}, U Susanto\textsuperscript{2}, and H Aswidinnoor\textsuperscript{4}

\textsuperscript{1}Plant Breeding and Biotechnology, Graduate School of IPB, Bogor, 16680, Indonesia, \textsuperscript{2}Indonesian Center for Rice Research, Subang, 41256, Indonesia
\textsuperscript{3}Indonesian Centre for Agricultural Biotechnology and Genetic Resources Research and Development, Bogor, 16111, Indonesia
\textsuperscript{4}Department of Agronomy and Horticulture, IPB, Bogor, 16680, Indonesia

E-mail: sitares_trias@yahoo.com

Abstract. In deep-water areas, the water depth increases gradually throughout the year and maintains up to more than 50 cm of deep of water for long period. In these situations, elongation ability is necessary to allow the plants to keep up with rising floodwater. The elongation of internode during submergence is regulated by environmental and hormonal factors. The objective of this study was aimed to identify the SNP markers on 384 SNPs linked with agronomical and morphological traits related to stagnant flooding tolerance. The research were conducted at Indonesian Center for Rice Research and Indonesian Centre for Agricultural Biotechnology and Genetic Resources Research and Development. The phenotypical data was collected from F2 from bi-parental crossing of IR 42 and IRRI 119. IR 42 was sensitive parent, and IRRI 119 was tolerant. DNA extraction for rice was using a modified version of Murray and Thompson method using cetyl tri-methyl-ammonium bromide (CTAB). The genotyping was carried out using 384 SNPs Golden Gate Illumina assay. Association analysis between SNP markers and phenotypical data was performed using General Linear Model in Tassel versus 5.0 software program. Based on GLM analysis, the significant marker for plant height with P value < 0.05 are TBGI275345, TBGI275367, and TBGI424383. The significant marker for number of tiller are TBGI000722, TBGI258600, TBGI270843, TBGI271066, TBGI271076, TBGI272122, TBGI272241, and TBGI327790. Two of them, TBGI424383 and TBGI271066 were expected associated with family of protein kinase which play role in plant stress signalling.

1. Introduction

Rice has a unique ability to elongate its internode with increasing water depth. During submergence, rice promotes growth of shoots toward the air-water surface which facilitates gas exchange with atmosphere [1]-[2]-[3]-[4]. Stagnant flooding at depths greater than 25 cm adversely affects the growth of rice in medium-deep areas (< 100 cm), even though the plants are not fully submerged. In particular, it reduces tillering and increases lodging [5]-[6]. The grain yield-related rice traits are controlled by multiple genes which are significantly influenced by environment [7]-[8]. If the amount and distribution of favourable genes are unknown, it is difficult
to apply the rice germplasm in conventional breeding. There are interaction between genes/QTLs underlying yield-related traits and particular ecological environments. For instance, the efficiency of certain favourable genes can be enhanced by proper environments, but countered by unsuitable environments [9]-[10]. Thus, it is important and prerequisite to study favourable trait-related genes in specific ecological environment for utilizing the rice germplasms.

Several studies have been conducted on the inheritance of early elongation ability [11]-[12]-[13]. The results differed from cross to cross, apparently because rice is traitized by a variety of phylogenetic backgrounds [14]. Two quantitative trait loci (QTLs) that control the early elongation ability on chromosomes 3 and 12 in F2 population. Another study performed a diallel analysis of early elongation ability showed that some additional recessive allele(s) might confer the earlier elongation [15].

Single nucleotide polymorphisms (SNPs) can be converted into genetic markers that are scored in mapping populations using various high-throughput SNP-typing technologies [16]-[17]-[18]. High-throughput SNP discovery [19]-[20]-[21]-[22]-[23]-[24] and genotyping technologies have simplified the generation of genetic maps and the analysis of recombinants [25]. Dense maps in economically important crops will be invaluable for marker-assisted selection programs [26], analyzing linkage disequilibrium [27], detection of intra-species cis-regulatory variation [28], and other quantitative genetic studies [29].

The elongation of internode, as well as the degree of elongation during submergence is regulated by environmental and hormonal factors viz ethylene, GA, and ABA [3]. Further, reference [30] reported that this traits is controlled by two major SK genes driven by the rice actin promoter. There are few reports available that specify the pattern of internode elongation under deep-water. However, very scanty report are available on response of physiological (leaf area, photosynthesis, and non-structural carbohydrates) and morphological (shoot length, tillers, culm thickness, spikelet fertility, shoot biomass, and grain yield) traits under deep-water rice [31]-[32]-[33].

The objective of the study is to select markers associated with agronomical traits of rice related to stagnant flooding tolerance using SNPs marker

2. Methodology

Analysis was conducted in Indonesian Centre for Rice Research (for DNA extraction) and Molecular Biology Laboratory of Indonesian Centre for Agricultural Biotechnology and Genetic Resource (for genotyping assay) on February to June 2016. Genetic materials used are population of P1 (IR 42), P2 (IRRI 119), and F2.

As markers, we used 384 SNPs linked with morphological and agronomical traits. All markers used were validated by Rice Genome Division of Indonesian Center for Agricultural Biotechnology and Genetic Resource [34]. Phenotypic data associated with the genotypic data were plant height, number of tiller, weight of 100 grain, panicle length, and grain yield. Panicle length, weight of 100 grain, and grain yield were selected based on plant height and number of tiller is the character that can be used as selection criteria for stagnant flooding tolerance. Plant height and number of tiller are characters related to stagnant flooding tolerance based on [31]-[35]-[36]-[37]. Weight of 100 grains, panicle length, and grain yield were determined as selection criterion for stagnant flooding tolerance. Background selection method was used in this experiment. There are two steps for genotyping, namely DNA extraction and Genotyping Assay.

2.1 DNA extraction

DNA extraction for rice was using a modified version of a method developed by Murray and Thompson [38]. It is a simple effective method utilizing cetyl tri-methyl-ammonium bromide (CTAB).

Materials used were 2-3 inches leaves of 3-week old plant (one leaf per sample), scissors, sterile 2 ml micro tubes, steel balls, liquid nitrogen, micropipettes, tips and tubes, water bath, centrifuge with rotor, -20 °C freezer, 800 µl 2x CTAB buffer warmed to 65 °C, 0.8 ml chloroform-isoamyl alcohol
(24:1), 600 µl isopropanol, 70 % ethanol, 200 µl TE buffer, 2 µl RNase (10 mg/ml), 20 µl (1/10 volume solution) sodium acetate, 400 µl (2 volumes) absolute ethanol.

DNA of genotype was bulked based on phenotypic clustering. Technique of sampling which will be used is bulked segregant analysis. Bulked segregant analysis (BSA) has been proposed as an efficient strategy for identifying DNA markers linked to the genes or genomic regions of interest [39]. BSA has been successfully used in rice for identifying large effect QTLs linked to rice yield under severe drought stress [40]-[41].

Dilution will be done to obtain the required concentration of DNA in Genotyping Assay. DNA diluted by ddH2O up to DNA concentration is suitable with its concentration for Genotyping Assay. Dilution formula is V1 x V2 x M1 = M2, with V1 is DNA volume before dilution, M1 is DNA concentration before dilution, V2 is final volume dilution, and M2 is concentration DNA for Genotyping Assay, that is 25 ng/mL.

2.2 GoldenGate Genotyping Assay

GoldenGate Genotyping Assay consisted of two processes: pre-amplification and post-amplification. Pre-amplification consisted of DNA activation, precipitation, resuspension, ASE (Allele Specific Extension), MEL (Master mixed Enzymatic Extension and Ligation), PCR plate preparation, and Inoc PCR. DNA activation process of each samples used random biotinylated with added MS1 reagent. Thus precipitation was done by adding PS1 reagent and 2-propanol and was continued resuspension by adding RS1 reagent. ASE process was by adding biotinylated gDNA and query oligos, hybridization reagent, and paramagnetic particle. Then it was continued with washing the product of non-specific hybridization and oligo exceed by adding AM1 and UB1 reagent. Then process of enzymatic extension and ligase was done by adding MEL master mix to DNA samples.

Preparation of PCR plate for amplification was by adding Titanium Taq DNA polymerase, and then sample was removed to PCR plate. Extension and ligase process were adding PCR (loc PCR) using 3 universal primer (reagent MMP), i.e. two primers with fluorescence labeled (Primer 1 and Primer 2), and 1 Primer biotinylated (Primer 3), where Primer 3 was tag the PCR product and to elute DNA strand which contained fluorescence signal. Then eluted sample was transferred from ASE plate to PCR plate.

Post amplification process consisted of PCR, binding PCR, INT plate for BeadChip, hybridization, washing BeadChip, and visualization of BeadChip on IScan system. PCR process was amplification of PCRA plate with fluorescence label, then was continued with binding PCR. Binding PCR was done by adding MPB reagent to PCR plate and then the suspension was transferred to plate filter. DNA strand was biotinylated with paramagnetic particle so that produced immobile double strand PCR product. In the INT plate for BeadChip step, PCR product with SS fluorescence was washed with UB1, MH1 and NaOH 0.1N and then was dissolved in intermediate (INT) plate. BeadChip was hybridize using Hyb Chamber for one night in illumina hybridization oven at 60°C to 45°C. BeadChip was transferred and washed using PB1 (2x) and XC4 (1x). Visualization of BeadChip on IScan Reader system use laser to excited Fluor form single-base extension product in bead. Light emission from the Fluor was recorded as picture with high resolution. The visualization product was analyzed to determining SNP genotype using Illumina’s BeadStudio Gene Expression Module [42].

2.3 Data analysis

Phenotypical data used were plant height and number of productive tiller of P1, P2, and F2 population. A number of 5% of minimum and maximum value of plant height and number of tiller from F2 population was analysis using t test to confirm the phenotypic variability. Association analysis between SNP marker and phenotypical data was tested using General Linear Model in Tassel 5 software [43]. Values of the Q matrix obtained in Structure were presented as covariates. The P value determines whether a marker was associated with phenotype and R² for marker evaluates the magnitude of QTL effect to phenotype.
3. Result And Discussion

DNA was bulked based on cluster analysis in phenotypic data of plant height and number of tiller. Plant height is the main character to avoid plant from stagnant flooding stress or escape strategy [44]. Length of stem was identified determine the stagnant flooding tolerance. Stem length have high correlation with plant height. Meanwhile, number of tiller is character which responsive to stagnant flooding stress [45]-[35]-[37]. Both of these characters can be observed at the end of the vegetative stage. Therefore, cluster analysis was performed on the characters. Others characters observed in the generative stage or physiological maturity stage were panicle length and weight of 100 grains.

We used the T-test to determine whether there is phenotypic variability observed in F2 population (Table 1). T test was performed on plant height and number of tillers. T test showed that that the average of plant height and number of productive tiller across 10% of minimum values (left extremes) were significantly different from those across 10% of maximum values (right extremes) (Figure 1 and 2).

| Table 1. T test of minimum and maximum value of plant height and number of tiller from F2 population |
| Variable       | N | Mean | StDev | SE Mean | P-Value |
|----------------|---|------|-------|---------|---------|
| Plant height   |   |      |       |         |         |
| PH_min         | 10| 123.1| 1.37  | 0.43    | 0.000   |
| PH_max         | 10| 161.0| 4.55  | 1.40    |         |
| Number of tiller |  |     |       |         |         |
| PT_min         | 10| 2.7  | 1.06  | 0.33    | 0.000   |
| PT_max         | 10| 18.3 | 1.06  | 0.33    |         |

Marker which showed polymorphic on 53 rice sample were 82 of 384 SNPs marker used. Association mapping analysis using GLM showed that there were five SNPs associated with plant height with probability value <0.05 and $R^2$ was ranged between 12-13%. Likewise, mapping association between the markers and the number of productive tillers showed 10 marker have probability value < 0.05 and $R^2$ was ranged between 6-15% (Table 5.2). Association mapping for panicle length showed 9 SNPs markers were significant with probability < 0.01 and $R^2$ was ranged 18-
28 %. $R^2$ showed magnitude of QTLs effect controlling the phenotype. Although it was detected with less statistical power, MAF<0.05 but > 0.02 is still appropriate to help in identification of smaller set of candidates genes using bi-parental population [46].

Based on General Linear Model (GLM) association analysis, significant genomic regions for plant height and number of tiller were identified based on P value < 0.05. The significant markers for plant height were TBGI275345, TBGI275367, dan TBGI424383. TBGI275367 was expected on same position with LOC Os06g09630 which probably related with 3-oxoacyl-synthase gene. LOC Os06g09630 were functionally annotated as chloroplast precursor putatively expressed genes on the basis of the homology with the previous annotated genes. Chloroplasts have been observed to be involved in light perception and interact with photoperiodic development acting as major light harvesting organs [47]. TBGI424383 is located in chromosome 11 and was mapped near from probably gene controlling serine protease. Serine protease have been shown to be involved in diverse processes regulating plant development and defence response to abiotic and biotic stress [48]-[49]-[50]-[51].

The significant SNPs for number of tiller were TBGI000722, TBGI258600, TBGI270843, TBGI271066, TBGI271076, TBGI272122, TBGI272241, and TBGI327790. The SNPs marker TBGI271066 is associated with OsWAK61 gene. The Wall-Associated Kinase (WAK) is a subfamily of receptor-like kinase (RLK), with some members identified as associated to cell wall, suggesting these genes are strong candidates to act as sensors directly linking the extracellular environment to the cytoplasm and triggering intracellular signals. TBGI327790 was associated with Glucan endo-1,3-beta glucosidase. Glucan endo-1,3-beta glucosidase protein that were up regulated under stress [52].

Proteins involved in multiple pathways showed significant changes in expression in response to stress environment, including oxidative stress response proteins, beta-glucanases, protein; kinases, glycine, and photosynthesis proteins. Signal transduction is an important molecular response which plays a major role in protecting the plants by modulating the stress-responsive gene expression. These signals may be transmitted to neighbouring cells/plants by emitting volatile organic compounds [53]. TBGI270843 was allegedly in same position with LOC_Os06g04810.1which is associated with leucine rich repeat protein gene.

### Table 2. SNP marker significantly associated with plant height and no of tiller under stagnant flooding stress

| Trait/marker | Chr | Position | P value | $R^2$ | Nearby gene candidate                      |
|--------------|-----|----------|---------|-------|------------------------------------------|
| Plant Height |     |          |         |       |                                          |
| TBGI275345   | 6   | 4869776  | 0.036   | 0.129 | -                                        |
| TBGI275367   | 6   | 4897700  | 0.038   | 0.130 | 3-oxoacyl-synthase, chloroplast precursor|
| TBGI424383   | 11  | 6199852  | 0.037   | 0.128 | Serine protease-like proteins             |
| No. of tiller|     |          |         |       |                                          |
| TBGI000722   | 1   | 421982   | 0.031   | 0.113 | Express protein                           |
| TBGI258600   | 5   | 25247484 | 0.039   | 0.106 | Glucose 1 dehydrogenase (GlcDH)          |
| TBGI270843   | 6   | 2096700  | 0.038   | 0.107 | Leucine rich repeat protein, putative, expressed |
| TBGI271066   | 6   | 2229688  | 0.034   | 0.110 | OsWAK61 - OsWAK receptor-like protein kinase |
| TBGI271076   | 6   | 2234192  | 0.018   | 0.129 | OsEF3                                    |
| TBGI272122   | 6   | 2829380  | 0.044   | 0.067 | -                                        |
| TBGI272241   | 6   | 2877413  | 0.01    | 0.152 | -                                        |
| TBGI327790   | 7   | 21148555 | 0.034   | 0.110 | Glucan endo-1,3-beta glucosidase precursor |
The most significant marker for the characters was described with QQ plot (Figure 3). QQ plot is a visualization of Table 5.1. Significant marker was showed by highest Log 10(P-value). The SNP marker associated with number of tiller, TBGI272241, which located in chromosome 6 at 2877413bp. TBGI2241 is not yet uncharacterized, so that its gene has not been identified yet. This provides an opportunity to identify a new gene associated with number of tiller in relationship with stress water tolerance.

![Figure 3. Quartile-quartile plot (qq plot) determines marker-traits association compare to the expected result](image)

The ability of multiple linear regression model to explain proportion of phenotypic variance could be used to identify simple assay involving a small number of SNPs which are designed for selecting lines with favourable alleles for target traits [46]. A linear model with two candidate marker explained 13.3 % of phenotypic variance for number of tiller. Two marker involving the regression model for number of tiller were TBGI270843 and TBGI272241 (Table 3 and 4). Meanwhile for plant height, regression model could explained 10 % of variance, which were contributed by SNPs marker TBGI258567 and TBGI275345.

| Source          | DF | Mean Square | Pr(> F) | Adj R² |
|-----------------|----|-------------|---------|--------|
| Number of tillers |    |             |         |        |
| Model           | 2  | 88.211      | 0.012   | 0.133  |
| Error           | 48 | 18.227      |         |        |
| Total           | 50 |             |         |        |
| Plant height    |    |             |         |        |
| Model           | 2  | 243.629     | 0.027   | 0.102  |
| Error           | 49 | 62.525      |         |        |
| Total           | 51 |             |         |        |
Table 4. Parameter Estimates

| Variable   | Estimate | Std. Error | Pr(>|t|) |
|------------|----------|------------|---------|
| Tiller number |         |            |         |
| Intercept   | 9.58     | 0.72       | 0.000   |
| TBGI270843  | -5.37    | 1.85       | 0.006   |
| TBGI272241  | 3.92     | 1.43       | 0.008   |
| Plant height |         |            |         |
| Intercept   | 145.47   | 2.6        | 0.000   |
| TBGI258567  | 5.01     | 2.69       | 0.068   |
| TBGI275345  | -4.82    | 2.4        | 0.050   |

4. Conclusion
Based on General Linear Model (GLM) association analysis, the significant marker for plant height with P value < 0.05 were TBGI275345, TBGI275367, and TBGI424383. The significant marker for number of tiller were TBGI000722, TBGI258600, TBGI270843, TBGI271066, TBGI271076, TBGI272122, TBGI272241, and TBGI327790. TBGI424383 and TBGI271066 probably associated with family of protein kinase which play role in plant stress signaling.

References
[1] Kende H K E and Cho H T 1988 Plant Physiol. 118 1105-10
[2] Sauter M 2000 Natur wissen schaften. 87 289-03.
[3] Vriese W H, Zhou Z and Straeten D 2003 Ann Bot. 91 263-70.
[4] Jackson M B and Ram P C 2003 Ann Botan. 91 227-41
[5] Singh S and Singh R K 2000 Rainfed Rice: A Source Book of Best Practices and Strategies in Eastern India (Los Banos, Philippines. International Rice Research Institute)
[6] Tuong T P, Kam S P, Wade L, Pandey S, Bouman B A M and Hardy B 2000 Traitizing and Understanding Rainfed Environments (Los Banos, Philippines. International Rice Research Institute)
[7] Huang R Y, Jiang L R, Zheng J S, Wang T S, Wang H C, Huang Y M and Hong Z L 2013 Trends Plant Sci. 8 218-26
[8] Ikeda M, Miura K, Aya K, Kitano H and Matsuoka M 2013 Curr. Opin Plant Biol. 16 213-20
[9] Liu G F, Yang J and Zhu J 2006 Acta Genet Sin. 33 607-16
[10] Sreedhar S, Reddy T D and Ramesha M S 2011 Int J Plant Breeding Genet. 125 1303-12
[11] Tripathi R S and Rao M J B 1985 Euphytica. 34 875-81.
[12] Suge H 1987 Jpn. J. Genet 62 69-80
[13] Eiguchi M, Hirano H Y, Sano Y and Morishima H 1993 Jpn. J. Breed. 43 135–39.
[14] Glaszmann J C 1987 Theor. Appl Genet. 74 21-30.
[15] Nemoto K, Ukai Y, Tang D Q, Kasai Y and Morita M 2004 Theor. Appl. Genet 109 42-7
[16] Gabriel S and Ziaugra L 2004 Curr. Protoc. Hum. Genet 60 2.12.1 - 2.12.18
[17] Gunderson K L, Steemers F J, Lee G, Mendoza L G and Chee M S 2005 Nat. Genet. 37 549–54
[18] Hui L, DelMonte T and Ranade K 2008 Curr. Protoc. Hum. Genet. 56 2.10.1-2.10.8.
[19] Marth G T, Korf I, Yandell M D, Yeh R T and Gu Z 1999 Nat. Genet. 23 452–6
[20] Weckx S, Del-Favero J, Rademakers R, Claes L and Cruts M 2005 Genome Res. 15 436–42
[21] Zhang J, Wheeler D A, Yakub I, Wei S and Sood R 2005 PLoSComput. Biol.1 e53
[22] Barbazuk W B, Emrich S J, Chen H D, Li L and Schnable P S 2007 Plant J. 51 910–8
[23] Li H, Ruan J and Durbin R 2008 Genome Res. 18 1851–8.
[24] Li R, Li Y, Fang X, Yang H and Wang J 2008 Genome Res. 19 1124–32
[25] Shifman S, Bell J T, Copley R R, Taylor M S and Williams R W 2006 PLoS Biol. 4 e395
[26] Prigge V, Melchinger A E, Dhillon B S and Frisch M 2009 Theor. Appl. Genet. 119 23–32
[27] Kruglyak L 2008 Nat. Rev. Genet. 9 314–8
[28] Stupar R M and Springer N M 2006 Genetics 173 2199–210
[29] Cookson W, Liang L, Abecasis G, Moffatt M and Lathrop M 2009 Nat. Rev. Genet. 10 184–94
[30] Hattori Y, Nagai K, Furukawa S, Song X J, Kawanr, Sakakibara H, Wu J Z, Matsumoto T, Yoshimura A, Kitano H, Matsuoka M, Mori H and Ashikari M 2009 Nature 460 1026–30
[31] Amante M M 1986 Evaluation of rice breeding lines under medium-deep (25-50 cm) water conditions (Thesis) (Gregorio: Aranante University)
[32] Sakagami J I, Joho Y and Ito O 2009 Ann. Bot. 103 171-80
[33] Vergara G V, Nugraha Y, Esguerra M Q, Mackill D J and Ismail AM 2014 Aob PLANTS 6 plu055
[34] BB Biogen 2015 Pengembangan TOOL Kits Sistem Deteksi Karakter Penting Tanaman Padi Unggul. Tim Genom Padi (Laporan Hasil Penelitian) (Bogor : Balai Besar Penelitian Bioteknologi dan Sumberdaya Genetik Pertanian)
[35] Singh S, Mackill D J and Ismail A M 2011 Field Crops Res. 121 311-23
[36] Collard B C Y, Septiningsih E M, Das S R, Carandang J J, Pamplona A M, Sanchez D L, Kato Y, Ye G, Reddy J N, Singh U S, Iftekhuruddaula K M, Venuprasad R, Vera-Cruz, Mackill D J and Ismail A M 2013 SABRAO J. Breed. Genet. 45 42-56
[37] Kato Y, Collard B C Y, Septiningsih E M, and Ismail A M 2014 Aob PLANT 6 plu058
[38] Murray M G and Thompson W F 1980 Nucleic Acids Res. 8 19
[39] Brauer M J, Christianson C M, Pai D A and Dunham M J 2006 Genetics 173 1813–16
[40] Quarrie S A, Lazic-Jancic V, Kovacevic D, Steed A and Pekic S 1999 J. Exp. Bot. 50 1299–306
[41] Venuprasad R, Dalid C O, Del Valle M, Zhao D, Espiritu M, Sta Cruz M T, Amante M, Kumar A and Atlin G N 2009 Theor. Appl Genet. 120 177–90
[42] Illumina 2008 Retrieved from http://support.illumina.com/documents.
[43] Bradbury P J, Zhang Z, Kroon D E and Bucker E S 2007 Bioinformatics. 23 2633-2635
[44] Hattori Y, Nagai K and Ashikari M 2011 Curr. Opin. Plant Biol. 14 100-5.
[45] Ismail AM, Johnson DE, Ella ES, Vergara GV, and Baltazar AM 2012 Aob PLANTS 2012 pls019
[46] Suwarno W B, Pixley K V, Palacios-Rojas N, Kaeppler S M and Babu R 2015 Theor Appl. Genet. 128 851-64
[47] Lepistö A, Kangasjärvi S, Luomala E M, Brader G, Sipari N, Keränen N, Keinänen M and Rintamäki E 2009 Plant Physiol.149 1261–76
[48] Nakagami H, Pitzschke A and Hirt H 2005 Trends Plant Sci. 10 339-46.
[49] Schaller A 2004 Planta 220 183-197
[50] Adam Z, Adamska I, Nakabayashi K, Osterstzer O, Haussuhl K, Manuell A, Zheng B, Vallon O, Rodermel S R, Shinozaki K and Clarke A K 2001 Plant Physiol. 125 1912-8
[51] Palma J M, Sandalio L M, Corpas F J, Romero-puertas M C, McCarthy I, Rio L A 2002 Plant Physiol. Biochem. 40 521-30
[52] Chen X, Dong Y, Yu C, Fang X, Deng Z, Yan C and Chen J 2016 PloS One 11 1-16
[53] Bruinsma J J, Schneider D L, Davis D E and Kornfeld K 2008 Genetics.179 811-28.

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