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Genetic Mapping of QTLs for Physiological Traits in Rice (*Oryza sativa* L.) by using Danteswari/Daggad Deshi Ril Population

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A B S T R A C T

Drought is a major constraint in rice- growing areas of Asia. Identification of genomic regions containing QTLs governing drought tolerance traits is inevitable for developing novel genotypes with enhanced drought tolerance. A RIL population of 122 lines derived from a cross of Daggaddeshi/Danteswari was used for identification of QTL governing traits associated with yield was used in the study. A new QTL (qDTY12.2) linked to grain yield was identified in both stress and non-stress conditions. Several QTLs linked to different secondary traits associated with grain yield in stress condition were also identified. These QTLs can be used for further studies using marker assisted breeding for enhancing drought tolerance.

Introduction

Rice is the predominant food crop for more than three billion of the world's population and contributes up to 80% of the daily calories' intake, specifically in Asia. Because of its semi-aquatic nature, smaller root system rice is severely affected by drought (Sahebi *et al.,* 2018). Millions of lowland rainfed areas in Asia are adversely affected by drought stress, which results in a drastic reduction in crop productivity by 13-35%. Water stress can arise in early growth stages of the crop; from flowering to grain filling, depending on the duration and intensity of stress (Wade *et al.,* 1999). Development of rice cultivars with augmented drought tolerance is thus pivotal in boosting production, strengthening yield stability, and allaying poverty in communities contingent on rainfed production.

Conventional breeding comprises of induced mutation, intergeneric and interspecific crosses. The availability of genetic variation in a mapping population, the selection criteria and the availability of proper selection protocol defines the achievement of a breeding program. The selection of parents based on criterions set by the breeding program plays an imperative role in the

successful development of the mapping population. The mapping population developed from the crosses between drought tolerant genotypes and high yielding drought susceptible genotypes has been shown to be efficient in development of high yielding cultivars with enhanced drought tolerance. Aside from this, the utilization of popular genotypes in the target environment as recipient parents provides an opportunity to create new genotypes with favourable traits associated with yield and in a region and inclination of the farmers, and thus increasing chance of approval of the novel cultivars.

Contemporary advancements in the field of plant physiology has resulted in the development of new and efficient techniques to enhance drought tolerance in plants (Oladosu *et al.,* 2019). Grain yield has been used as the selection criteria for superior cultivar under drought conditions owing to the low heritability and large influence of genotype by environment interaction, however this has been proved to be inefficient as the (Bolanos *et al.,* 1993). As the time passed by, the selection based on physiological characters has been the focus of conventional breeding as these traits are less time consuming and reliant on genetic variation. The efficacy of molecular biology in selecting the pivotal gene sequences, introgression or genetic transformation these QTLs strongly depends on the knowledge of the physiological processes which determine the yield of a plant (Kirigwi *et al.,* 2007; Araus *et al.,* 2002). Significant attempts to target the secondary traits have been made since many years (Jongdee *et al.,* 2002). An ideal secondary trait is (i) genetically correlated with grain yield under drought stress (ii) have high heritability (iii) durable and plausible to measure (iv) not linked to loss of yield under ideal growth conditions (Edmeades *et al.,* 2001). The study presented below was conducted to map the QTLs governing different physiological factors linked to grain yield under drought stress.

Materials and Methods

Planting materials

Daggaddeshi, a drought tolerant *indica* landrace, which has deep and robust root system is adapted to rainfed upland and Danteswari, a drought susceptible low land *indica*eco type (Chand *et al.,* 2016) with long slender grain and good head rice recovery was used in the study. These two parental lines are well adapted to rainfed target population environments (TPE) in Chhattisgarh and differ for a range of traits. A mapping population of 122 Recombinant Inbred Lines (RILs) was developed from the cross of Danteswari x Daggaddeshi.

Field trials

The trial was conducted was conducted in the experimental fields of Department of Plant Molecular Biology and Biotechnology, IGKV, Raipur (C.G) during the wet season in the year of 2017 and 2018 (July-December). The F14 RILs along with the parents were planted in split plot and RCBD design were evaluated under three different conditions; irrigated, rainfed and terminal stage drought (TSD) at the experimental farm, Department of plant molecular biology and biotechnology, IGKV, Raipur (21^0 16' N and 81^0 36' E at altitude of 289.6 meter above sea level), C.G. The trial was conducted in RCBD with two replications under irrigated, rainfed and TSD. The seed rate was maintained at $2.5g/m^2$ for transplanted conditions and $6g/m^2$ under direct seeding for rainfed trial. The experiments were conducted in sandy or clay loam inceptisols with a pH ranging from 6.8-7.4 and organic carbon of 0.32-0.34%. For irrigated field, a puddled condition was created where water was allowed to standby

from sowing/transplanting to ten days before maturity whereas for rainfed trial, the fields were never irrigated and the rainwater was drained after the rain to make a quick appearance of drought, thereby keeping the fields free from standing water throughout the season. Sowing and transplanting for TSD was delayed by 20-25 days so as to coincide with the dry spells to induce the reproductive stage stress after the termination of monsoon. Proper package of practices was followed to raise a good crop.

Rainfall

During 2017, there was a total of 584 mm rainfall during the cropping season 2017. The crop was germinated and established following the rainfall received in late June. Significant reduction in rainfall was observed during tillering stage. There were 9 continuous rainless days during this stage. As of 2018, there was a total of 966.2 mm rainfall with 10 continuous rainless days during tillering (Fig. 1).

Field phenotyping

Plants from each F14 families were assessed for agronomic trait, plant height (measured in centimetres from the soil surface to the tip of the tallest panicle), panicle length, grain yield and biological yield (grain yield + straw yield). The measurements were taken following the guidelines by Standard Evaluation System for Rice (IRRI, 1996).

DNA extraction and SSR polymorphism

The genomic DNA was isolated from the parents and the RILs using the CTAB method (Gawel and Jarret, 1991). The extracted DNA content was quantified using Nano Drop® ND-1000 Spectrophotometer and parental polymorphism studies were conducted through 162 SSR markers. PCR mix for one reaction (volume 20µL) contained 2µL DNA, 13.5µL sterile and nanopore water, 10X assay buffer, 1µL dNTP, 0.5µL of each forward and reverse primers and 0.5µL Taq DNA polymerase. PCR amplification was performed with the following steps: pre-denaturation at 94ºC for 4 minutes, followed by 35 cycles of 94 ºC for 1 minute, 55 ºC for 1 minute and 72 ºC for 2 minutes and last step for 5 minutes at 72 ºC. Amplified products were analysed using 5% polyacrylamide gel. Electrophoresis was carried out for 1 hour at 199 volts and the gel along with the DNA sample was obtained with ethidium bromide (10µg/10ml) for 40-45 minutes. Gel was visualized on UV trans-illuminator and image was observed on a computer screen (Molecular Imager®, Gel doc ™ XR system 170-8170, BIO-RAD, USA).

The genetic diversity between the breeding parents are evaluated using polymorphism. A total 830 microsatellite markers were used to detect the polymorphism, out of which 162 markers were polymorphic. These selected polymorphic markers were employed to genotype the F14 RIL population.

Results and Discussion

Analysis of variance

Analysis of variance was done for grain yield in both the years for split plot design (Table 1). The mean sum of square for environments were found to be significant in both the years, indicating that the environmental conditions were different from one another. At 0.01 probability, the genotype x environment interaction was also significant proving the differential response of the genotypes to environment. Mall *et al* (2012) has also reported significant genotype x environment interaction under water stress. Each environment was analysed individually under Randomized Complete Block Design (RCBD)

(Gomez and Gomez,1984) since genotype x environment interaction is significant. The genotypes were also observed to be significant in each environment (Table 2).

Genotyping of population

830 microsatellite primers were screened for the purpose of genotyping, out of which 162 primers were found to be polymorphic and they displayed 19.52% polymorphism. Out of these 162 markers, 73 (45.06%) showed 1:1 segregation 1% level of significance in$\chi 2$test, and the others presented skewed distribution towards either of the parents. More female alleles (86.1%) and less male alleles (12.3%) were formed by RM171 whereas RM277 produced a greater number of male alleles (83.6%) and a smaller number of female alleles (11.5%). High A: B ratio was exhibited by RM171 (7.0). Cai *et al.*, (2011) has also reported such skewed marker distribution. Based on genotypic data, GGT2.0 was used to analyse the rate of integration of the parents into the lines. The data analysed for high yielding lines by GGT2.0 showed that a major QTL region on chromosome 1 which was contributed from female parent and from chromosome 3 by male parent can be used for the selection of desirable lines (Fig 2).

Table.1 Analysis of variance for grain yield (gram/m^2) under split plot design

Source of variation	Degree of freedom	Mean sum of square	
		Wet season - 2017	Wet season - 2018
Replication	1	150993	55812.7
Environments (a)	4	4476531.89*	5592899.3**
Error (a)	4	454621.5	209812.05
Genotypes (b)	120	103525.04**	73383.98**
a x b	480	36989.03**	15237.47**
Error (b)	600	12998.25	7198.4

*= significant at 0.05 probability level **=significant at 0.01 level

Table.2 Analysis of variance for grain (g/m^2) under RCBD design

Analysis of variance	Mean sum of square, wet season-2017			
Source of variation	Degree of freedom	Irrigated	Rainfed	TSD
Replication	1	1789348.58*	31898.8*	23658.9**
Genotypes	121	119679.2**	117658.2**	13125.95**
Error	121	43425.03	39858.08	2125.66
	Mean sum of square, wet season-2018			
	Degree of freedom	Irrigated	Rainfed	TSD
Replication	1	569548.65**	131456.79**	159852.45**
Genotypes	121	55982.68**	29855.12**	99855.4**
Error	121	15980.5	8291.69	2280.81

Table.3 QTLs for traits linked to grain yield under stress condition for wet season 2017 and 2018

Condition	QTL	Chromosome	Marker interval	LOD	R^2	Additive effective
Rainfed(2017)	*qDTY1.1*	1	RM486-RM14	4.6	12.5	45.6
	qDTY9.2	9	HvSSR9-19 – HvSSR9-25	3.5	9.5	-36.89
Rainfed (2018)	*qDTY1.2*	1	HvSSR1-2 – HvSSR1-49	3.8	7	-42.88
	qDTY7.1	7	HvSSR7-40 – HvSSR 7-43	3.5	12.2	58.05
Irrigated (2017)	*qDTY1.2*	1	RM499 – HvSSR1-24	3.6	13.2	89.8
Irrigated (2018)	*qDTY 12.2*	12	RM20 – HvSSR12-35	4	9.2	20.1
TSD(2017)	*qDTY12.2*	12	HvSSR12-48 – RM260	3.7	10	22.52
	qDTY1.3	1	RM24 to RM449	3.5	9.8	-30.21
	qDTY3.3	3	RM7 – RM232	5	12.2	28.8

Table.4 QTLs linked to secondary traits linked to grain yield under stress condition

Condition	Trait	QTL	Chromosome	Marker interval	LOD	R^2	Additive effective
Rainfed	Plant height	*qPH1.2*	1	RM1-HvSSR1-87	4.5	16	85.2
	Plant height	*qPH1.3*	1	RM84-HvSSR1-87	7.1	25	-29.88
	Plant height	*qPH1.4*	1	RM475-RM221	3.5	9	-19.8
	Panicle length	*qPL1.2*	1	RM1-HvSSR1-87	4	15	15.8

Fig.1 Daily rainfall pattern during wet season-2017 and 2018

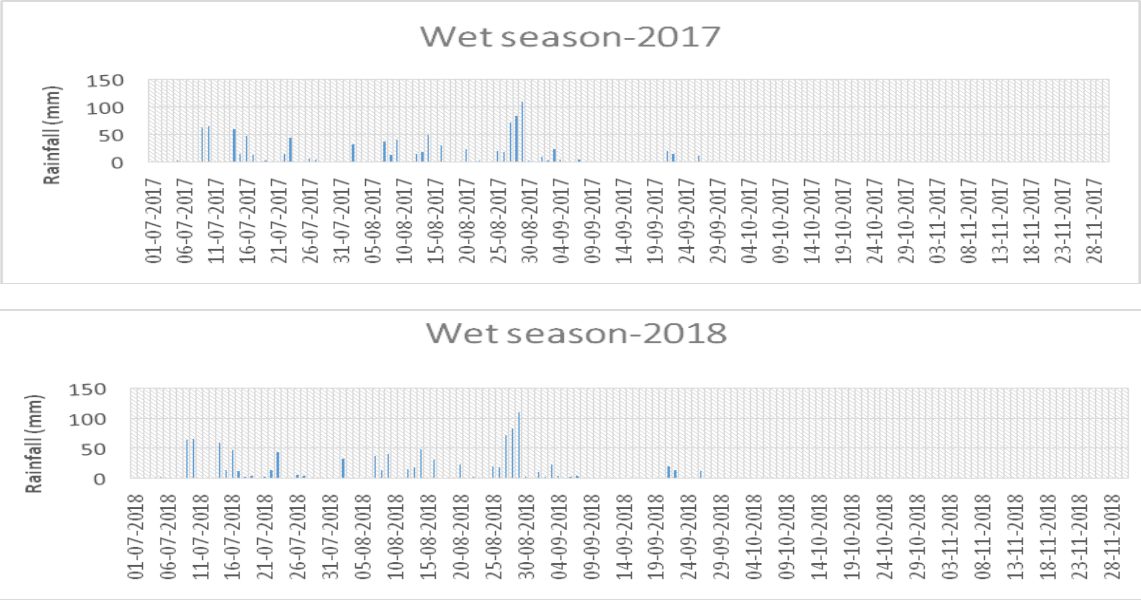
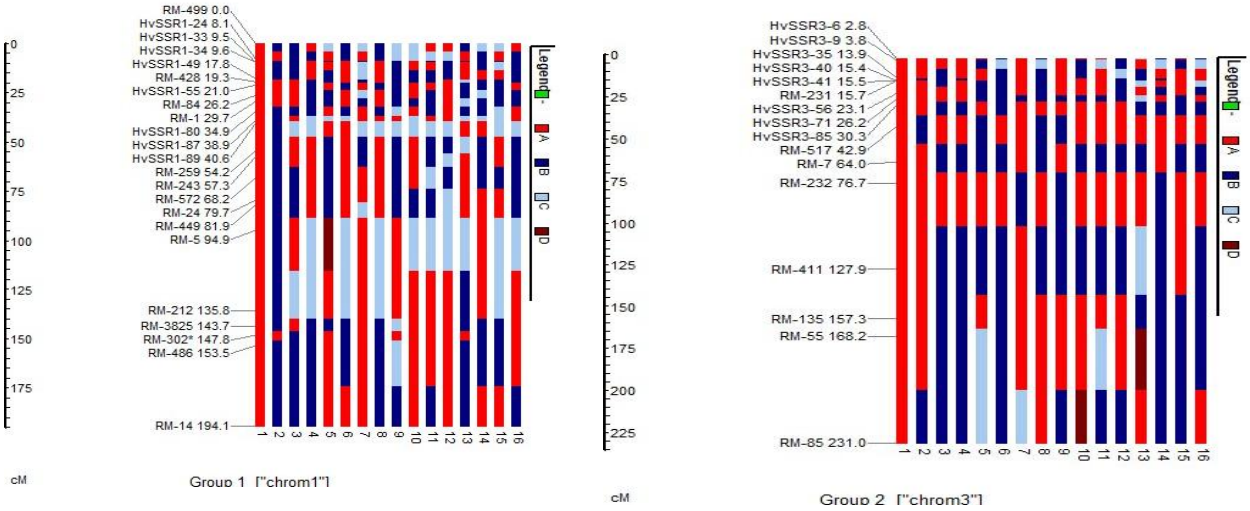


Fig.2 Graphical genotype of chromosome 1 and 3 of Danteswari x Daggaddeshi showing expected proportion of introgression



Identification of QTLs

The analysis of genetic data along with phenotypic data in QTL Cartographer 2.5 identified four. QTLs for grain yield under stress conditions (Table 3).*qDTY1.1* on chromosome 1 was found to be linked to grain yield under rainfed condition. This QTL lies between RM486-RM14 with a LOD score of 4.6 and a phenotypic variation of 45.6%. The QTL has a positive additive effect indicating that the alleles for grain yield under stress condition comes from the donor parent, Daggaddeshi.*qDTY1.1* was also reported earlier to be linked to grain yield under reproductive stage drought in rice (Vikram *et al.,* 2011; Ghimire *et al.,* 2012). *qDTY7.1* (HvSSR7-40 – HvSSR 7-43) was identified to be linked to grain yield under rainfed condition and this marker had an LOD score of 3.5 and a phenotypic variation of 58.05%. Sandhu *et al.,* 2017 reported this QTL to have a positive effect on grain yield under drought. Another QTL identified to be associated with grain yield under stress with a positive additive effect is *qDTY3.3* (RM7-RM232) which has a LOD score of 5 and phenotypic variance of 12.2%. Yadav *et al.,* 2019 has also reported *qDTY3.3* to be linked to grain yield under drought stress. No novel QTLs with positive additive effect for grain yield were identified. Other QTLs for grain yield under drought stress with negative additive effects were *qDTY9.2, qDTY1.2, qDTY12.2, qDTY1.3. qDTY1.2* was identified to be linked to grain yield in both controlled (irrigated) and rainfed conditions; it had a positive additive effect in controlled conditions whereas it exhibited a negative additive effect in rainfed condition. Sandhu *et al.,* 2014 reported this QTL to be linked to grain yield under drought in IR64/Kali Aus RIL population.*qDTY9.1*is reported to be associated with grain yield under drought in Adays el/IR64 RIL population (Singh *et al.,* 2016).*qDTY9.2* and q*DTY12.2*were not reported earlier. A new QTL with positive additive effect was identified in stress and non-stress trials between HvSSR12-48 – RM260 with a LOD score of 3.7 and phenotypic variance of 10%.

QTLs for secondary physiological traits linked to grain yield were also mapped using QTL Cartographer 2.5 for wet season 2018 (Table 4). Three QTLs for plant height and one QTL for panicle length were identified in rainfed trials. A QTL for plant height (*qPH1.2*) with LOD score 4.5 and phenotypic variance 16% under rainfed condition was identified with positive additive effect. *qPH1.2* was reported earlier to be linked to plant height in BC_2F_8 population of Swarna/IRGC81848. *qPH1.2*, *qPH1.3*, *qPH1.4*were identified by Prince *et al.*, (2000) to be in C813-RZ909 interval where semi-dwarfing locus sd-1 was reported.

In conclusion the consistent QTL for grain yield under irrigated and rainfed conditions on chromosome 12 was identified. This QTL can be used for marker assisted selection for drought tolerance in rice. Further studies can be conducted to use this QTL for fine mapping or gene pyramiding in local drought tolerant genotypes.

Abbreviation: QTL: Quantitative trait loci, RIL- Recombinant Inbred Line

References

Araus, J. L., Slafer, G. A., Reynolds, M. P., and Royo, C. 2002. Plant breeding and drought in C3 cereals: what should we breed for? *Ann. Bot.* 89: 925–940.

Bolaños, J.; Edmeades, G.O. Eight cycles of selection for drought tolerance in lowland tropical maize. II.Responses in reproductive behaviour. Field Crops Res. 1993, 31, 253–268.

Cai J, Zhang X, Wang B, Yan M, Qi Y, Kong L (2011) A genetic analysis of segregation distortion revealed by molecular markers in *Lophopyrumponticum*chromosome 7E. J Genet. 90: 373-376.

Edmeades, G. O., Bolanos, J., Lafitte, H. R., Rajaram, S., Pfeiffer, W., and Fischer, R. A. 1989. Traditional approaches to breeding for drought resistance in cereals. In: *Drought Resistance in Cereals*, pp. 27–52, Baker, F. W. G., Eds., CAB International, Wallingford, Oxon, UK.

Gawel N.J & Jarret R.L (1991). A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea.* Plant Molecular Biology Reporter 9:262–266.

Ghimire, Krishna &Quiatchon-Baeza, Lenie& Vikram, Prashant & Swamy, Mallikarjuna& Dixit, Shalabh& Ahmed, Helaluddin& Hernandez, Jose & Borromeo, Teresita & Kumar, Arvind. (2012). Identification and mapping of a QTL (qDTY1.1) with a consistent effect on grain yield under drought. Field Crops Research. 131. 88-96. 10.1016/j.fcr.2012.02.028.

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research. 2nd edn. John Wiley & Sons, New York.

Gulab Chand, R.K. Bajpai, Shashikant Sahu, Madan Prasad Paikra, Himanshu Patel (2016). Varietal performance of rice for their yield and its attributes in farmers field of Durg. Asian Journal of Bio Science. 11:241-243.

IRRI (International Rice Research Institute). Standard evaluation system for rice. International Network for Genetic Evaluation of Rice (INGER). Manila (Phillippines): IRRI. 1996; 51.

Jongdee, B., Fukai, S., and Cooper, M. 2002. Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice. *FieldCrops Res.* 76: 153–163.

Kirigwi, F. M., Van Ginkel, M., Brown-Guedira, G., Gill, B. S., Paulsen, G. M., and Fritz. A. K. 2007. Markers associated with a QTL for grain yield in wheat under drought. *Mol. Breed*. 20:

401–413.

MalathiSurapaneni, Divya Balakrishnan, Sukumar Mesapogu, Krishnam Raju Addanki, Venkateswara Rao Yadavalli, Tripura VenkataV. G. N., SarlaNeelamraju (2017). Identification of Major Effect QTLs for Agronomic Traits and CSSLs in Rice from Swarna/*Oryza nivara* Derived Backcross Inbred Lines. *Frontiers in Plant* 8:1027.

Mall AK, Swain P, Singh ON, Baig MJ (2012) Use of genotype × environment interactions and drought susceptibility index for identification of drought tolerant genotypes at vegetative stage in upland rice. Indian J Dryland Agric Res Dev. 27: 73-78.

Nitika Sandhu, Shalabh Dixit, Mallikarjuna Swamy B.P, Prashant Vikram, ChallaVenkateshwarlu, Margaret Catolos, Arvind Kumar (2018). Positive interactions of major-effect QTLs with genetic background that enhances rice yield under drought. Scientific Reports. 8:1626. DOI:10.1038/s41598-018-20116-7.

Nitika Sandhu, Anshuman Singh, Shalabh Dixit, Ma Teresa Sta Cruz, Paul Cornelio Maturan, Rajinder Kumar Jain, Arvind Kumar (2014). Identification and Mapping of Stable QTL With Main and Epistasis Effect on Rice Grain Yield Under Upland Drought Stress. BMC Genetics. 2014 May 27; 15:63.

Prashant Vikram, BP Mallikarjuna Swamy, Shalabh Dixit, Helal Uddin Ahmed, Ma Teresa Sta Cruz, Alok Kumar Singh, ArvindKumar (2011). *qDTY* $_{1.1,}$ a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genetics* volume 12, 89 (2011).

Price AH, Steele KA, Moore BJ, Barraclough PB, Clark LJ (2000) A combined RFLP and AFLP linkage map of upland rice (*Oryza sativa* L.) used to identify QTLs for root penetration ability. TheorAppl Genet 100: 49–56.

Renu Singh, Yashi Singh, Suchit Xalaxo, S Verulkar, Neera Yadav, Shweta Singh, Nisha Singh, K S N Prasad, K Kondayya, P V Ramana Rao, M Girija Rani, T Anuradha, Y Suraynarayana, P C Sharma, S L Krishnamurthy, S K Sharma, J L Dwivedi, A K Singh [5], P K Singh, Nilanjay, N K Singh, Rajesh Kumar, S K Chetia, T Ahmad, M Rai, P Perraju, Anita Pande, D N Singh, N P Mandal, J N Reddy, O N Singh, J L Katara, B Marandi, P Swain, R K Sarkar, D P Singh, T Mohapatra, G Padmawathi, T Ram, R M Kathiresan, K Paramsivam, S Nadarajan, S Thirumeni, M Nagarajan, A K Singh, Prashant Vikram, Arvind Kumar, E Septiningshih, U S Singh, A M Ismail, D Mackill, Nagendra K Singh (2016). From QTL to Variety-Harnessing the Benefits of QTLs for Drought, Flood and Salt Tolerance in Mega Rice Varieties of India Through a Multi-Institutional Network. Plant Science Jan; 242:278-287.

Sahebi, M.; Hanafi, M.M.; Rafii, M.Y.; Mahmud, T.M.M.; Azizi, P.; Osman, M.; Miah, G. Improvement of drought tolerance in rice (Oryza sativa L.): Genetics, genomic tools, and the WRKY gene family. BioMed Res. Int. 2018, 2018, 3158474.

ShaileshYadav, NitikaSandhu, Vikas KumarSingh, MargaretCatolos, Arvind Kumar(2019). Genotyping-by-sequencing based QTL mapping for rice grain yield under reproductive stage drought stress tolerance. Scientific Reports 9:14326.

Wade, L.J., Amarante, S.T., Olea, A., Harnpichitvitaya, D., Naklang, K., Wihardjaka, A., Sengar, S.S., Mazid,

M.A., Singh, G. and McLaren, C.G., 1999. Nutrient requirements in rainfed lowland rice. Field Crops Res. 64: 91-107.

YusuffOladosu, Mohd Y. Rafii, Chukwu Samuel, AroluFatai, Usman Magaji, Isiaka Kareem, Zarifth Shafika Kamarudin, Isma'ila Muhammad, Kazeem Kolapo (2019). Drought Resistance in Rice from Conventional to Molecular Breeding: A Review. Int. J. Mol. Sci. 2019, 20, 3519

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