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Selected lines from Tai Nguyen Cho Dao for drought tolerance and good grain quality in rice (*Oryza saltiva.L*) at Long An , VietNam

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Abstract**—** *Selected of Tai Nguyen Cho Dao (TNCD)rice varieties tolerant to drought, high-yielding and good quality is very essential demands has raised to provide poor farmers in coastlines conditions as lowland in Long An ,VietNam. The purpose of this experiment is to selected of 22 lines from TNCD rice varieties tolerant to drought on the basis of a combination of two methods by molecular markers and evaluated phenotype. Evaluating drought tolerance of 22 lines from TNCD on the basis in the field of drought conditions to select promising lines to meet for farmers applying into production.* ***Methods:*** *selection of drought tolerant alleles for landrace rice at Long An by SSR(simple sequence repeated) markers and evaluated phenotype.* ***Results:*** *Some high yielding and good drought tolerant lines as lines: (TNCD 01, TNCD 15 and TNCD 22), however, also many lines failed due to many reasons: expansion of growth day, high rate of unfilled grain, not good for grain quality. Three lines (TNCD01, TNCD15, TNCD22) good for three characters such as high yield, and tolerance with drought. Based on morphological and SSR marker the line TNCD 01, TNCD 22 was identified as good quality which was further confirmed through molecular characterization techniques using RM201, HATRI13D, RM328, RM316, RM 5353, RM3480 primers. Sequence of TNCD01; TNCD22 were submitted to GenBank with accession number MT992254 and MW917241 respectively. Tolerant lines from Tai Nguyen Cho Dao have high-yielding rice varieties. This is a opportunity to improve good landrace rice varieties for condition of selected drought landrace rice varieties in Long An ,Vietnam.*

Keywords**—** ***high-yielding, grain quality, drought tolerance, landrace, SSR molecular marker.***

I. INTRODUCTION

Landraces have been shown to be excellent sources of genes for novel alleles (Loresto et al., 2000). In VietNam, rice is the major agricultural export, especially Tai Nguyen Cho Dao(TNCD) rice is landrace varieties . The cooked kernels of TNCD rice have a highly prized scent and texture. Tai Nguyen Cho Dao rice(TNCD) is normally grown in the Souther of VietNam , based on rain with limited irrigation and acid sufate- soild at Long An. Therefore, it is always affected by drought stress, leading to the reduction in growth and yield. Drought stress affects plant morphology, physiology, and molecular mechanisms. Upon drought stress, cell turgor pressure is decreased due to low water potential in cells. This causes a decrease in the relative water content, leaf water potential, stomatal conductance, and transpiration rate (Siddique et al 2001). Drought-resistant rice plants consume less water indicative of increased root biomass events under conditions of re-irrigation. The HDR gene with AP2/ERF transcoding factor, isolated in the mutant lineage of Arabidopsis (functionally attached), controls root strength trait, branching, epidermal cells, leaf thickness with elevated chloroplast proportions in mesophyll cells, promoting photosynthesis assimilation and photosynthesis performance (Karaba et al. 2007) .Simple sequence repeat is an important tool for genetic variation identification of

germplasm (Ma et al., 2011). SSR marker have some merits such a quickness, simplicity, rich polymorphism and stability, thus being widely applied in genetic diversity analysis, molecular map construction and gene mapping (Ma et al., 2011), construction of fingerprints (Ma et al., 2011), genetic purity test (Ma et al., 2011), analysis of germplasm diversity (Ma et al., 2011, Lang et al 2021) utilization of heterosis, especially in identification of species with closer genetic relationship. A total of 18,828 Class 1 di-, tri- and tetra-nucleotide SSRs, representing 47 distinctive motif families, were identified and annotated on the rice genome. An abundance of microsatellite markers is now available through the published high-density linkage map; there was an average of 51 hyper variable SSRs per Mb, with the highest density of markers occurring on chromosome 3 (55.8 SSRMb-1) and the lowest occurring on chromosome 4 (41.0 SSRMb-1) (IRGSP 2005).In particular, Wang et al. (2007) compared gene expression between water and shallow rice varieties under drought stress, using cDNA microarray. Shallow rice varieties IRAT109, Haogelao, Han 297 and water rice varieties Zhongzuo 93, Yuefu, Nipponbare were used. After reading the DNA sequence, there were 64 unique ESTs expressed at high levels in shallow rice varieties and 79 in water rice varieties. The author predicts that the expression of high levels of target genes in shallow rice may improve drought stress tolerance in rice and other closely related crops (Wang et al. 2007).This study is also need further selection and identification of drought tolerant varieties, good shape and high yield which need attention for TNCD at VietNam**.**

II. MATERIALS AND METHODS

The experiment is carried out parents from 22 different lines from Tai Nguyen Cho Dao. The yield of all the 22 lines were similar to the yield of the standard checks.

Phenotype analysis

A field experiment was transplanted to an irrigated lowland field in a randomized complete block design in three replications in the field of *High Agricultural Technology Research Institute for Mekong delta*.Vietnam (HATRI) at Binh Thuy, Can Tho. One hundred lines TNCD selectd with 22 lines from TNCD with KhaoDawMali 105, Tau Binh (Checked) were used to evaluated agronomic characteristics and drought detection through sensory test and genotypic analysis using SSR markers in lab of HATRI. Data on important agronomic traits like plant height, panicle length, filled grains/panicle, unfiled grain/panicle, 1000-grain weight, harvest index and yield were recorded.

Ten randomly selected plants of each genotype were used for agronomic data analysis. Data on plant height (cm), number of effective tillers/plants, panicle length (cm), number of filled grains/panicle, 1000-grain weight (g), days to maturity and grain yield/plant (g) were recorded and subjected to statistical analyses using SAS software. After harvesting, the seeds of each genotype were dehulled for evaluation of the grain quality. The grains were classified into different types based on their dimension according to (Lang et al, 2018).

Phenotyping: Evaluation of 22 lines for drought tolerance

Screening at seeding stage

Screening of 22 lines for drought tolerance was done under controlled environment condition. Rapid screening method was used. Two pre-germinated seed were planted field . After three days, For drought treatment, the seeds were sown in pots containing vermiculite and nutritional soil, and seedlings were watered with tap water until they reached the three-leaf stage. The seedlings were used as experimental materials. The control seedlings continued to be watered, while water was withdrawn from the drought treatment seedlings for 30 days. Three replicates were performed for each treatment. All experiments were carried out in a greenhouse; the seedlings were harvested prior to measuring the physiological and biochemical indicators. The measured physiological and biochemical traits included leaf dying score and leaf-death score . The drought resistance indices of the physiological and biochemical indicators were calculated as:Modified standard evaluation score (Table 1) was used in rating the symptoms of drought . Scoring was done 30 days. At this period, 22 lines and checked for tolerance (Khoa DawMali 105) scored 1-3 and susceptible (Tau Binh) scored 9.

Table1. Modified standard evaluation score (SES) of visual drought injury at seedling stage(IRRI .1967. Lang et al, 2012).

Score	Observation
1	Normal growth, no leaf symptom
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled
5	Growth severely retarded; most leaves rolled; only a few are elongating
7	Complete cessation of growth; most leaves dry; some plants dying
9	Almost all plants dead or dying

Fig.1. A. growth conditions (drought), (Figure 1,B), under normal

Genotype, Quality Control

DNA extraction

The 22 lines/varieties(TNCD) were grown in pots, maximum protection was employed to ensure healthy and disease-free seedlings. The leaves were collected 2-3 weeks after planting for DNA extraction.Standard molecular grade chemicals and general techniques for preparing stock solutions, buffers, reagents, and equipment were followed according to (Sambrook *et al.*,1989). Molecular work was conducted at the Genetics and Plant Breeding Department of the High Agricultural Technology Research Institute for Mekong delta,Vietnam (HATRI).

DNA suitable for PCR analysis was prepared using a simplified procedure (McCouch *et al.*, 1988). A piece of a young rice leaf (2 cm) was collected and placed in a labeled 1.5 ml centrifuge tube in ice. The leaf was ground using a polished glass rod in a well of a spot test plate (Thomas Scientific) after adding 400 µl of extraction buffer. Grinding was done until the buffer turned green, an indication of cell breakage and release of chloroplasts and cell contents. Another 400 µl of extraction buffer was added into the well by pipetting. Around 400 µl of the lysate was transferred to the original tube of the leaf sample. The lysate was deproteinized using 400 µl of chloroform. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA was precipitated using absolute ethanol. DNA was air-dried and re-suspended in 50 µl of TE buffer (Lang, 2002).

DNA quality checks used 1% agarose by melting 3 g of agarose in 300 ml of TAE buffer. The mixture was heated in a microwave for 5-6 min and then cooled to around 55-60 0C. This was then poured on a previously prepared electrophoresis box with combs. Gels were prepared and the combs removed after about 45 min. Seven microliters of DNA sample plus 3 µl of loading buffer (Tris 1 M pH = 8.0, glycerol, EDTA 0.5 M pH = 8.0, xylene cyanol 0.2%, bromophenol blue 0.2%, and distilled water) was run at 70-80 v, 60 mA for 45 min or until the loading buffer dye moved far away from the wells. The gel was then taken out and stained with ethidium bromide, after which it was observed under UV light.(Lang et al 2015)

Microsatellite analysis

The whole microsatellite analysis included PCR assay, polyacrylamide gel electrophoresis, and band detection and scoring.

PCR assay

Microsatellite primers were used to survey polymorphism on the samples. These were randomly selected from the 6 microsatellite primer pairs currently available for rice such (Table 2). The PCR reaction was as follows:

Table 2. SSR marker on chromosome 9 and 8 used in the present study

SSR markers	Chromosome	Forward primer sequence	Reverse primer sequence
RM201	9	F -5'CTCGTTTATTACCTACAGTACC-3'	R -5'CTACCTCCTTTCTAGACCGATA -3'
RM328	9	F-5'AAGTTTGTACACATCGTATACA-3′	R-5'CGCGACCAGTACTACTACTA-3′
HATRI 13D	9	F- 5' caccacacacccattttcac -3'	R-5' cgcgagtggtgtcttctgt -3'
RM316	9	F-5' CTAGTTGGGCATACGATGGC -3'	R-5' ACGCTTATATGTTACGTCAAC -3′
RM5353	8	F-5' ACCCTCGATCTCCTAGGCTG-3'	R -5'TCTACTCCAAACCCATTGCC-3'
RM3480	8	F-5'GTGCCAAGGAGATTGGATTG -3'	R-5'ATGGTCTGCAACTCTGCAT G-3'

Reactions were overlaid with mineral oil and processed in a programmable thermal controller set for 35 cycles of 1 min at 94 ^{0}C, 1 min at 55 ^{0}C, and 2 min at 72 ^{0}C, with a final extension at 75 ^{0}C for 5 min. After amplification, 10 µl of stop solution was added to the PCR product, which was then denatured at 94 ^{0}C for 2 min. Eight microliters of each reaction were run on polyacrylamide gel.

Data Analysis

Analysis of variance: The agro-morphological data collected were initially analyzed through analysis of variance to verify genetic variation in the traits measured. The few traits with in significant genetic variation, based on the F-test, were not considered for further analyses.

III. RESULTS AND DISCUSSION

Evaluation of physiological responses of 22 lines TNCD under drought-stress conditions. Selected 22 lines and two checked (KDM 105 and Tau Binh) , were evaluated for drought tolerance by growing the seedlings in soil with 100% or in normal growth conditions all of the lines were similar (Figure 1A,B), but they differed under drought field capacity. 22 lines from NTCD displayed the most drought-tolerant phenotype, with the lowest leaf dying score. This was similar to the performance of KDM105(the drought-tolerant line chected). The highest leaf-death score was detected in lines TNCD under drought stress. These data suggest that lines : 1, 3 is the most drought-tolerant line, while some lines are the most susceptible(5-9 score) . The 22 lines are selected through continuous seasons in 2022 and selected under drought to select the stages of 22 lines to evaluate this generation the plant shapes which are relatively uniform were recorded. Through reviews with 22 lines tolernce to drought in the period recorded 1 lines for tolerance to drought such as line TNCD01, TNCD 15, TNCD 22 . Score tolerance numbers 3 such as TNCD 02 , TNCD 03, TNCD 11, TNCD 12, TNCD 13, TNCD 14, TNCD 16 and TNCD 20 the same with KDM 105(cheched). Remain lines are supceptible (Tau Binh Cheked). Continued assessment and analysis of yield and yield components. Response to drought stress in TNCD lines and KDML105, TauBinh , were compared for leaf death and leaf dying score) normal and drought-stress conditions. The mean + 1 standard error (SE) was derived from four replicates. (Table3).

Table 3. Drought tolerance score, of leaf of the extreme tails identified by screening drought (30 days)

STT	Lines	Drought score	leaf dying score **(%)**	leaf-death score **(%)**
1	Tau Binh (Checked)	9	5.533	7.41
2	KDM105(Checked)	3	0.579	0.82
3	TNCD01	1	0.06	0.00
4	TNCD02	3	0.612	0.96
5	TNCD03	3	0.976	0.50
6	TNCD04	9	7.75	6.32
7	TNCD05	5	1.745	3.59
8	TNCD06	5	1.632	2.62

9	TNCD07	5	1.745	2.43
10	TNCD08	5	1.669	2.12
11	TNCD09	7	5.756	5.02
12	TNCD 10	5	2.563	2.16
13	TNCD 11	3	0.75	2.32
14	TNCD 12	3	0.85	0.32
15	TNCD 13	3	0.17	0.32
16	TNCD 14	3	0.532	0.51
17	TNCD 15	1	0.062	0.05
18	TNCD 16	3	0.96	0.32
19	TNCD 17	5	0.612	2.96
20	TNCD 18	7	5.69	4.98
21	TNCD 19	5	1.422	3.89
22	TNCD 20	3	0.745	2.59
23	TNCD 21	7	7.632	4.62
24	TNCD 22	1	0.045	0.01

Line of 22 TNCD there are several applications in which DNA marker data may be useful for breeding, such as cultivar identity, assessment of genetic diversity (Collard and Mackill 2008). Screening drought gene is based on molecular marker (Lang et al 2015). Molecular values are assessed based on polymorphism targets and codominant genome on the varieties. To be based on information of genetic map of (Mackill et al 2006) recorded with the respective molecular marker with marker on chromsome 9. With 5 respective 22 lines have noted (Table 2) the difference between the recorded molecular markers with groups of SSR markers as follow: The line (3:TNCD01, 14:TNCD12, 15:TNCD13, 16:TNCD14, and TNCD22) has polymorphic with molecular markers of HATRI13D , RM201(figure 2A, 2B). The more sensitive method of QTL detection is the simultaneous analysis of the effects of markers binding in the same interval of chromosomes when studying genetic resistance to dry conditions. The effect of different regions on each chromosome on the 3 target traits (selection criteria) associated with drought resistance is shown in the relevant tables. Four drought SSR markers : RM 201 - RM 328 and RM 155- RM 511 have a very significant influence on the two target traits DR, YG and DF. Most target traits showed meaningful influence on defined regions of the genome but DR and DF traits showed influence within a distance of the genetic map (Lang et al, 2012.b) . This article is the first report on SSR based marker using TNCD landrace rice detected for drought tolerance. In the present study, 22 TNCD germplasms tested for genetic diversity were arbitrarily selected, therefore there is a possibility that they have a similar genetic backgrounds, which can result in relatively low genetic polymorphism. However, the fact that most of the primer sets produced normal PCR products is considered, SSR -based molecular markers developed in the present study could provide useful genetic information and materials for future rice drought tolerance breeding programs and genetic diversity study. The PCR product will be amplified with primer RM201, RM 328 , HATRI 13D, RM316 on chromosome 9(Lang et al., 2012.a) and RM5353, RM3480 (on chromosome 8 (Kanjoo et al.,2012) with the genetic code on 22 lines and drought sets to view polymorphisms of these lines (figure 2 and figure 3). TNCD lines evaluated with RM201 markers associated with DC(drought score) traits and for polymorphisms with bands of 210 bp 1:Tau Binh (Suceptible checked) and 225bp it's the same band as 2: KDM 105(drought cheked) . Similary , HATRI 13D to selectd plant homozygous for drought . Only 3: TNCD 01; 14:TNCD 12; 15:TNCD 13;16: TNCD 14 and 24:TNCD 22 give the same banding pattern ás KDM 105(190bp) and Tau Binh (210bp).

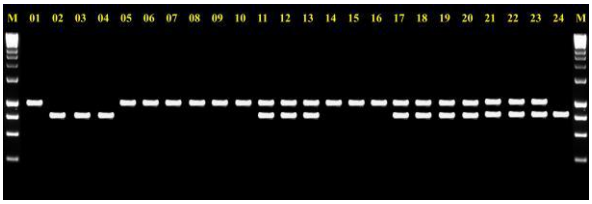

Figure 2A

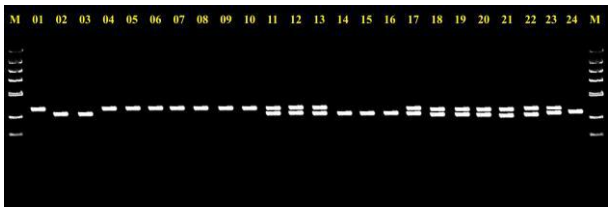

Figure 2B

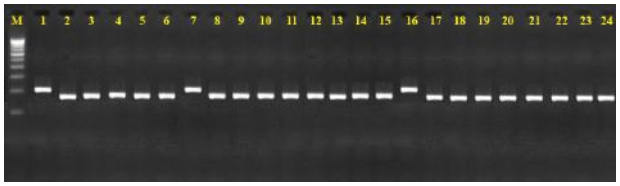

Figure 2C

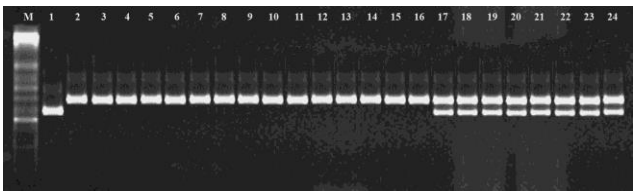

Figure 2D

Fig.2. PCR product of the line at RM 201(A) HATRI 13D (B) RM328 (C) RM316(D), the gene associated with tolerance to drought in chromosome number 9 position on the agarose gel 3%. With reference to the banding patterns of 1:Tau Binh(Susceptible) and 2:KDM 105 (resistance). 3-24 lines of TNCD

The maker RM5353-RM3480 linked with drought on chromosome 8 (Kanjoo et al .,2012) The selectd the plants with two markers in homozygous condition is shown in figure 3. A and figure 3.B.

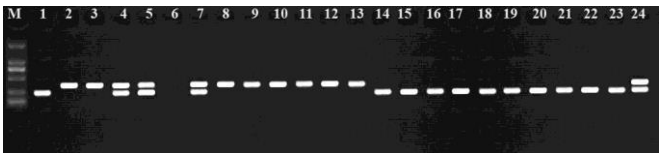

Figure 3A

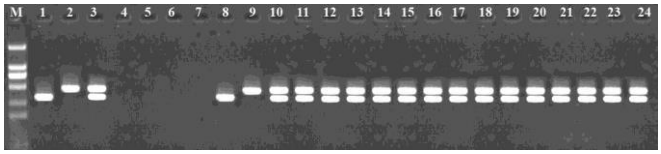

Figure 3B

Fig.3. PCR product of the line at RM5353(A), RM3480 (B) , the gene associated with tolerance to drought in chromosome number 8 position on the agarose gel 3%. With reference to the banding patterns of 1:Tau Binh(Susceptible) and 2:KDM 105 (resistance). 3-24 lines ofTNCD

Yield components

The promising lines of the TNCD variety are planted and assess the yield and yield components. The results showed that these lines had more equal and evener length panicle than the opposition, the lines were quite good panicle , the number of tilling / hill was quite good, and the ratio of panicle was average.Rice yield of grains,and 1000-grain weight . Most lines cultivars had more than 100 grains per panicle, and this character is desirable for breeding program. The 1,000-grain weight ranged from 25.21 to 28.78 g (Table 4). This is very important for farmers choise some size large and bold such as lines TNCD 01, TNCD0 6, TNCD 11 and TNCD 22. In terms of recorded productivity in the present study, yield grain from TNCD 01, TNCD 02, TNCD 11, TNCD 12, TNCD 15, TNCD 16 and TNCD 22 gives the highest yield over compare with KDM 105 and Tau Binh are (59.42 g/plant and 38.17g/ plant respectively), of which lines TNCD 01, TNCD 02, TNCD 11, TNCD 12, TNCD 15, TNCD 16 and TNCD 22 gives the highest yield (67.33;66.23; 66.48 ,63.60, 62.24, 65,73 and 86.03 g/plant) respectively. Through reviews with 22 lines tolerance to drought in the period recorded 6 lines for resistant to drought score 1 such as line TNCD 01, TNCD 15, TNCD 22, the same with KDM 105(cheched) . Through evaluation results of hight plant recorded only 4 lines TNCD 07, TNCD 10, TNCD 16, TNCD 18 had hight plant less than 140 cm. The number of grains per panicle is usually highly proportional to the spikelet number, such as TNCD 07 , TNCD 09 give good for spikelet / panicle (165.22;162.33 grain/panicle) respectively .

Table 4: yield and components yield of 22 lines from TNCD with two rice checked

acession	lines	Hight plant (cm)	Tilling/(Hill)	Weight of 1000 grain(g)	Filling/ panicle)	unfilling (%)	Length panicle (cm)	Biomass (g)	yield (g/ plant)	HI
Tau BInh	Tau Binh	169.33a	19.00d	26.69c	97.23f	17.95c	24.28	86.67c	38.17e	0.226c
KDM	KDM105	161.67a	16.33g	28.51a	127.67e	10.85e	24.22	65.67e	59.42c	0.316b
3	TNCD01	162.67a	19.12d	26.8c	140.44c	23.23b	24.94	86.67c	67.33b	0.401a
5	TNCD02	159.23b	16.67g	26.17c	145.56c	21.41b	27.44b	85.25c	66.23b	0.474a
16	TNCD03	158.67b	13.67h	27.23b	130.11d	27.22a	26.22c	61.67e	46.9d	0.278c
17	TNCD04	136.67d	17.27f	25.43d	100.78f	28.74a	25.46d	50.33f	41.14d	0.291c
18	TNCD05	160.67a	13.62h	25.32d	127.33e	26.79a	28.14a	53.33f	46.58d	0.335b
20	TNCD06	156.68b	17.11f	26.54c	115.23f	18.26c	27.32b	56.23f	32.25e	0.336b
25	TNCD07	162.23a	19.33d	26.25c	165.22a	12.83e	25.89d	57.67f	40.38d	0.350b
31	TNCD08	158.33b	19.67d	26.45c	108.44f	25.5a	26.25c	43.33g	27.5f	0.288c
32	TNCD09	166.33a	19.33d	26.31c	162.33a	16.01c	27.44b	46.67g	34.91e	0.277c
33	TNCD 10	132.33d	17.00f	26.44c	59.05g	20.45b	26.06c	45.67g	28.72	0.253c
34	TNCD 11	147.33c	20.67c	26.63c	137.89	24.97c	26.44c	85.12c	66.48b	0.450a
35	TNCD 12	154.25b	25.22a	25.22d	119.89f	19.01c	26.17c	100.00a	63.6b	0.238c
56	TNCD 13	152.14b	18.01e	25.72d	102.78f	26.92a	26.56c	83.33c	46.16d	0.214c
57	TNCD 14	153.67b	17.33f	26.23c	109.78f	22.91b	25.67c	80.00c	37.08e	0.202c
58	TNCD 15	150.33b	20.33c	26.24c	136.62d	12.71d	26.78c	81.67	62.24b	0.405a
59	TNCD 16	139.67d	21.67b	25.59d	155.44b	14.84d	25.33d	91.67b	65.73b	0.282c
65	TNCD 17	142.25c	19.33d	25.57d	133.44d	14.17d	25.17d	65.00e	39.04e	0.246c
69	TNCD 18	136.33d	19.32d	25.93d	67.67g	26.49a	25.06d	61.67e	19.08	0.147d
75	TNCD 19	148.33c	19.67d	25.32d	136.11d	11.76e	26.78c	76.67d	59.42c	0.279c
76	TNCD 20	154.16b	18.32e	25.41d	136.2d	15.21d	26.22c	62.23e	56.23c	0.254c
77	TNCD 21	152.23b	17.62f	25.21d	110.32f	14.25d	26.52c	62.54e	58.24c	0.321b
79	TNCD 22	155.32b	25.33a	26.06c	154.56b	14.82d	26.61c	90.56b	86.03a	0.421a

Evaluation of 22 lines for quality

Evaluation of rice quality appearance TNCD in physical characteristics, such as the length and width of the rice sample can play an important role in the willingness of consumers to pay for rice. When analyzing the size of rice grains is evaluated according to the IRRI standard scale. Tai Nguyen Cho Dao seed record size has a long rice grain size of 7.24-7.50mm rice grains . This is a very medium group of rice grains. Analysis of the chalkiness ratio of Tai Nguyen Cho Dao lines noted: most lines have chalkiness and chalkiness ratio in order (Table5).

Twenty two advanced lines along after checked with KhaoDawMali 105 and Tau Binh to quality analysis. Grain quality of rice consists of several components: the milling quality such as head rice (Lang et al 2005,Lang et al 2012b). Cooking and eating qualities are mostly determined by amylose content (AC), gelatinization temperature (GT), of the grain starch (Lang et al 2005). Appearance quality is mainly specified by grain shape as defined by grain length, grain width, the length–width ratio, and the translucency or chalkiness of the endosperm (Tang et al 1986). These traits are considered important for the ideal texture of cooked rice, especially for many rice consumers in South and Southeast Asia (Wand et al 2007). Brown rice percentage varied from 77.07-80.52%. The head rice percentage of lines ranged from 41.67-50.23%.Most of the lines were found to give chalkiness. Most consumers prefer rice with intermediate amylose

content ranged between 20-24%, compared to (KDM 105= 18.12%) (Table 5).

Table 5. Quality of 22 lines TNCD from Mua season 2022 at Can Duoc(Long An province)

Acession	lines	% brown rice	%white rice	% head rice	Length (mm)	L/W	gelatinization temperature (GT) (Score)	Chakiness (% score 9)	%Amylose
Tau Binh	Tau Binh	78.34d	75.39-c	41.73g	8.06 a	3.27c	3	6.67-e	24.63b
KDM	KDM105	77.07d	74.62-d	41.90g	8.86a	3.38b	7	0.00f	18.12e
3	TNCD01	79.38d	76.72 b	49.84b	7.19b	3.34b	3	8.33c	24.98b
5	TNCD02	79.32d	74.06-ef	49.47b	6.93c	3.46a	3	7.67d	25.67 a
16	TNCD03	79.38d	77.52a	44.15 f	7.60 b	3.34b	3	12.00b	24.63b
17	TNCD04	80.04a	77.65a	41.67g	7.19 b	3.33b	3	13.00 b	24.98b
18	TNCD05	78.04ab	73.72-f	43.19bc	7.15 b	3.32b	3	7.33 d	22.01d
20	TNCD06	77.63d	75.42c	47.38c	6.99c	3.26c	3	6.33-e	24.28b
25	TNCD07	79.86d	77.69a	44.55g	7.56b	3.20c	3	11.33 bc	24.00b
31	TNCD08	79.51d	74.84d	49.33b	7.32 b	3.44a	3	17.67a	23.35c
32	TNCD09	81.26b	76.32b	46.65d	6.96c	3.38b	3	8.00 c	22.12-f
33	TNCD 10	79.32d	75.62c	45.62e	7.19 a	3.34b	3	8.33c	24.98b
34	TNCD 11	78.23d	76.23b	49.23b	7.93 b	3.47a	3	7.67-d	25.67 a
35	TNCD 12	80.52c	76.25b	46.35d	7.30 b	3.34b	3	10.00 b	24.63b
56	TNCD 13	81.23b	76.77b	46.05d	7.09 b	3.33bb	3	13.00 a	24.98b
57	TNCD 14	79.23ab	75.73c	42.77g	7.05b	3.32b	3	7.33-de	22.01d
58	TNCD 15	80.21c	78.26a	50.23a	6.86 c	3.30b	3	7.00d	22.12d
59	TNCD 16	82.23a	76.25b	46.23d	7.19 b	3.31b	3	8.33c	24.98b
65	TNCD 17	80.15c	76.28b	46.95d	6.93c	3.35b	3	7.67d	25.67 a
69	TNCD 18	79.23d	75.73c	42.77g	7.80 b	3.34b	3	10.00b	24.63b
75	TNCD 19	82.20a	77.30a	42.80	7.09b	3.33bb	3	13.00 a	24.98-b
76	TNCD 20	80.14c	76.23b	45.62	7.05b	3.32b	3	7.33 d	22.01d
77	TNCD 21	80.23c	75.56c	46.23	7.12b	3.11d	3	7.23 d	22.17d
79	TNCD 22	82.15a	76.26b	50.23a	7.79b	3.23c	3	7.62d	23.12c

DNA profiles

The result of the amplification of the TNCD01 lines TNCD22 with RM 223 tershows the read able DNA bands measuring around 900-1250 bp. The success of amplification with PCR is evidenced by the process of sequencing the of *DNA* product with good quality . CLUSTAL 2.1 multiple sequence (https://www.genome.jp › tools-bin › clustalw) alignment compared MW917241(NTCD 22) with MT992254(Lang. 2021) with 84.295% . According to Miller et al. (1990) and Claveri and Notredame (2003), the higher score obtained the higher the homology of the two sequences, while the query coverage is a percentage of the long nucleotide aligned with the database in the BLASTn analysis(https://blast.ncbi.nlm.nih.gov/Blast.cgi). The E-value that gives statistically significant to both sequences.

The E-value indicates the homologous level between the lower sequences, whereas the lower of the E-value indicates that the two sequences are identical(MT992254(TNCD01) and accession number MW917241(TNCD 22). The similarity value of the Lines TNCD 22 cultivars is 92.27% which is similar to sequence, namely Rice in GenBank sequences of TNCD 01 have been registered in GenBank with accession number MT992254.

IV. DISCUSSION

Drought and molecular markers were used to marker on 22 lines at screening phase. An important aim may be to fix alleles in their homozygous state as early as possible. For example, pure lines landrace methods, screening is often performed at the TNCD01, TNCD 22 when most loci are homozygous. Using co-dominant DNA markers, it is possible to fix specific alleles in their homozygous state. However, this may require large population sizes; thus, in practical terms, a small number of loci may be fixed at each generation (Koebner & Summers 2003). An alternative strategy is to 'enrich' rather than fix alleles—by selecting homozygotes and heterozygotes for a target locus—within a population in order to reduce the size of the breeding populations required. For these results throught 4 markers with the homozygote the same with KDM 105 varieties and some allelels give heterozygote for contamination.To find the target breeding tolerant to drought and high yield.The rice varieties are grown and observed growth period of rice varieties. In the rice varieties evaluation recorded the varieties remained 8 lines good for drought . Grain yield in rice is a complex trait multiplicatively determined by its three component traits: number of panicles, number of grains per panicle, and grain weight, all of which are typical quantitative traits Yongzhong et al 2010

Table 3 : Evaluated yield and yield component of the varieties recorded 22 lines. Through evaluation results of yield and yield component recorded only 5 lines had high yield were line number TNCD 07, TNCD 10, TNCD 16, TNCD 18 and had hight plant day less than 140 cm (132.33;136.67,139.67, and 136.33 respectvitive). The number of grains per panicle is usually highly proportional to the spikelet number. To understand the making of the number of grains per panicle, it is essential to understand the basic biological processes of panicle development, as well as the differentiation of meristems into spikelet's at salinity in rice. From an agronomic perspective, the number of spikelet per panicle can be attributed to two components: the duration of panicle differentiation and the rate of spikelet differentiation (Huang Y et al 2006). This result gives 22 lines are selected through continuous seasons in 2022 and selected under drought to select the stages of 22 lines to evaluate this generation the plant shapes which are relatively uniform were recorded. Luo and Zhang 2001 divided drought resistance into 4 types including drought tolerance, drought escape, drought avoidance and drought recovery. Our experiment results indicated that **leaf dying score** and leaf-death score, and traits associated with drought avoidance.Therefore, the inheritance of drought tolerance and drought avoidance is closely correlated and interact with one another; they are not separated(Liguo et al 2016) .Through reviews with 22 lines drought to drought in the period recorded 1 for tolerance to drought such as line TNCD01, TNCD 15, TNCD 22 . Score tolerance numbers 3 such TNCD 02 , TNCD 03, TNCD 11, TNCD 12, TNCD 13, TNCD 14, TNCD 16 and TNCD 20 the same with KDM 105(cheched)(table 3) These lines also give good survival during 30 days with drought tolerance . As discussed above, there are PCR product of the line at RM 201,HATRI 13D, RM328 , RM3252 on chromosoe 9 and RM5353, RM3480 on chromosome 8 , the gene associated with tolerance to drought in chromosome number 9 and 8 for ladrace rice TNCD , Thís ís now over whelming evidence for the existence of extensive regions of conserved colinearity among cereal species at genetic map level. This knowledge can be exploited to advance marker studies on all grass species and to extend our knowledge of key syntenic agronomic genes as they are placed on pure lines in rice landrace .

V. CONCLUSIONS

Evaluating SSR on 22 different lines TNCD recorded different polymorphisms focus on chromosome 9, and 8 but difference group through screening 22 lines recorded a line tolerant to drought conditions during 30-days rate of survival of 95% which is 3 lines good for drought (TNCD 01, TNCD 15 and TNCD 22) . Some promising lines having good grain such as TNCD 01, TNCD 02, TNCD 11, TNCD 12, TNCD 15, TNCD 16 and TNCD 22 gives the highest yield (67.33;66.23; 66.48,63.60,62.24, 65,73 and 86.03 ; g/plant) respectively . Howver give good the high yield and drought tolerance with three lines (TNCD 01, TNCD 15, and TNCD 22) good for three characters such as high yield, and tolerance with drought and showing tolerance and seed multiplication is on going.

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