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Genetic diversity, population structure, marker validation and kinship analysis for seedling stage cold tolerance in *indica* rice

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ABSTRACT

Cold stress affects rice production globally. In India, around 4 million hectares of rice area are affected by seedling stage cold stress. A panel of 48 indica rice genotypes including all phenotype classes viz. very highly tolerant to susceptible ones were analyzed with linked SSR markers for kinship, population structure and genetic diversity for seedling stage chilling stress tolerance. High level of genetic diversity existed in the panel population. The heterogeneity in the panel population favored the presence of linkage disequilibrium. The population structure could differentiate the panel into two sub-populations separating the tolerant and susceptible lines into separate sub-groups. A lower value of alpha (α=0.0589) indicated that the trait had a common primary ancestor. The three phenotypic groups i.e. susceptible, moderately tolerant and tolerant to very highly tolerant groups were clearly separated out by kinship and cluster analysis. The donor lines in the panel exhibited the presence of multiple QTLs as well as different QTL combination in different lines for the expression of tolerance to cold stress. These tolerant genotypes can be used as donor lines in breeding programs to incorporate different combination of cold stress tolerance QTLs. The molecular markers used in the study were observed to be robust one to identify the highly tolerant, moderately tolerant and susceptible genotypes. Hence, these markers will be useful for marker-assisted breeding programs.

Key words: *Genetic diversity, kinship, population structure, seedling stage cold stress tolerance*

INTRODUCTION

Cold weather threatens rice production in 24 countries of the world covering around 15 million hectares of rice area. In India, the high altitude areas, northern and north eastern parts are mainly affected by low temperature stress. Indian rice cultivation faces mainly seedling stage cold stress followed by subsequent high temperature at flowering stage (Pradhan et al., 2016). Boro and part of dry season rice of India spreading over around 4 million hectares are affected by such stress subsequently reducing the yield to a considerable extent. The germination process and seedling growth require an optimum temperature ranging from 25-35^{0}C. When this temperature is reduced to 15^{0}C or below these processes are highly affected (Nakagahra et al., 1997). This may result in poor germination, slow seedling growth, withering, yellowing, reduced tillering and stunted growth (Zhang et al., 2005; Andaya and Tai, 2006; Lou et al., 2007; Suh et al., 2010; Pradhan et al., 2015). The frequency and extent of such incidence of low temperature stress has been increased across several regions of Asia due to the climate change (Pradhan et al., 2015; Pradhan et al., 2016).

Cold stress tolerance is a complex trait governed by multiple genes/QTLs. Around 30 QTLs were reported to be responsible for cold stress tolerance in rice (Kwak et al., 1984; Nagamine, 1991; Misawa et al., 2000; Qian et al., 2000; Kim et al., 2000; Qu et al., 2003; Andaya and Mackill, 2003; Fujino et al., 2004; Zhang et al., 2005; Andaya and Tai, 2006; Jiang et al., 2006; Han et al., 2007; Lou et al., 2007; Jiang et al., 2008; Koseki et al., 2010; Wang et al., 2011; Suh et al., 2012; Kim et al., 2014). Pyramiding of these QTLs in superior high yielding background can help overcoming the problem under the changing climate scenario. But lack of availability of robust markers for the QTLs and strong donors having many QTLs responsible for the trait in single background are major limitations for

marker-assisted breeding program. To overcome this problem, screening and identification of strong donors is a necessary step. There are reports on screening and identification of cold tolerant rice genotypes (Kwak et al., 1984; Nagamine, 1991; Misawa et al., 2000; Qian et al., 2000; Kim et al., 2000; Qu et al., 2003; Andaya and Mackill, 2003; Fujino et al., 2004; Zhan et al., 2005; Zhang et al., 2005; Andaya and Tai, 2006; Jiang et al., 2006; Han et al., 2007; Lou et al., 2007; Jiang et al., 2008; Koseki et al., 2010; Wang et al., 2011; Suh et al., 2012; Kim et al., 2014; Pradhan et al., 2015; Pandit et al., 2017). The genetic diversity in rice is usually studied by using SSR molecular markers (Wu and Tanksley, 1993; Xiao et al., 1996; Panaud et al., 1996; Olufowote et al., 1997; Thanh et al., 1999; Herrera et al., 2008; Pervaiz et al., 2010; Das et al., 2013; Babu et al., 2014 and Pradhan et al., 2016). The robust markers that can distinguish different genotypes for their cold stress tolerance needs to be identified. Also, the genetic diversity among parental lines should be assessed before selection of suitable parental lines in a breeding program.

In the present investigation, we have assessed the 48 *indica* rice lines for their genetic diversity, population structure using 33 linked SSR markers for identification of suitable donor lines to be used in breeding programs for developing chilling stress tolerant high yielding lines. Also, robust markers identified can be used in marker-assisted breeding programs for cold tolerance in rice.

MATERIALS AND METHODS

Materials

Forty eight *indica* rice genotypes were used in the present study comprising all phenotypic classes of cold stress tolerance *i.e.,* very highly tolerant, highly tolerant, tolerant, moderately tolerant and susceptible (Pradhan et al., 2016; Pandit et al., 2017). The list of genotypes is presented in Table 1.

DNA isolation and selection of SSR markers

Leaf samples were collected from 21 days old seedlings of 48 genotypes (Table 1). Total genomic DNA of the genotypes were isolated using liquid nitrogen for grinding using CTAB extraction buffer (100mM Tris-HCl pH 8, 20mM EDTA pH 8, 1.3M NaCl, 2% CTAB) and chloroform-Isoamyl alcohol extraction followed by RNAase treatment and ethanol precipitation (Murray and Thompson 1980). The DNA samples were diluted to approximately 30ng/µL after estimation of DNA concentration. Thirty three linked SSR markers were used to select the seedling stage cold tolerance.

PCR amplification and visualization of markers linked to seedling stage chilling stress

Polymerase chain reaction was performed by taking 20 µl aliquot using 1.5mM Tris HCL (pH 8.75), 50mM KCL, 2mM $MgCl_2$, 0.1% TrotonX-100, 200µM each of dATP, dCTP, dTTP, dGTP, 4pmole of each forward and reverse primers (Table 2), 1 unit of Taq polymerase and 30ng of genomic DNA. A Programmable thermal cycler was used for amplification of genomic DNA samples (Veriti, Applied BioSciences). First, the reaction mixture was denatured for 4 min at 94^0C and then continued to 35 cycles of 1 min denaturation at 94^0C, 1 min annealing at 55^0C, 1 min extension at 72^0C; and then a final extension for 10 mins at 72^0C. Agarose gel of 2.5% containing 0.8 g/ml Ethidium Bromide was used for electrophoresis. Aliquots of 10 µl of the products from PCR amplification were loaded in 2.5% in 1X TBE (pH 8.0). Size of amplicons was determined by the 50bp DNA ladder. The gel was run at 60 volts (2.5V/cm) for 4 hours and photographed using a Gel-Doc System (SynGene).

Genetic diversity and population structure

Data were scored on the basis of presence or absence of the alleles for each genotype-primer combination. PowerMarker Ver3.25 program was used for data analysis to generate number of alleles, allele frequency, gene diversity, heterozygosis and polymorphic information index (PIC) (Lu et al., 2005). STRUCTURE 2.3.4 software a model based approach was used for data analysis to obtain possible population structure (Pritchard et al., 2000). The model choice criterion to detect the most probable value of K was ΔK, an ad hoc quantity related to the second-order change of the log probability of data with respect to the number of clusters inferred by STRUCTURE (Evanno et al., 2005). Structure Harvester was used for estimation of the ΔK value as function of K showing a clear peak as the optimal K value (Earl and Von, 2012). Principal Coordinate analysis and unrooted tree

construction was done by using DARwin5 software (Perrier and Jacquemoud-Collet, 2006).

RESULTS AND DISCUSSION

Genetic relatedness/ kinship by principal co-ordinate and cluster analyses

Cluster analysis was carried out to assess genetic distance and genetic relatedness using UPGMA method. The tree constituted three major clusters (Fig. 1) of which cluster I can be grouped as the tolerant to very highly tolerant, whereas cluster II and III can be grouped as moderately tolerant and susceptible ones, respectively. Cluster I consisted 16 genotypes accommodating all the tolerant to very highly tolerant genotypes (red encircled) in the tree except Chakhaopspoireitol which is moderately tolerant to chilling stress (Fig. 1). The moderately tolerant lines are mainly observed in cluster II along with three susceptible types (green encircled). This cluster included 19 genotypes of which 16 are moderately tolerant and 3 are susceptible to chilling stress. Similarly, the cluster III contained all the sensitive genotypes to chilling stress along with three moderately tolerant genotypes (pink encircled). The principal coordinate analysis (PCoA) separately placed the tolerant and susceptible genotypes in four different quadrants (Fig. 2). All the tolerant, highly tolerant and very highly tolerant genotypes were placed in the 2nd and 3rd quadrant (red encircled) except Bamak and Geetanjali that were placed along with all the moderately tolerant

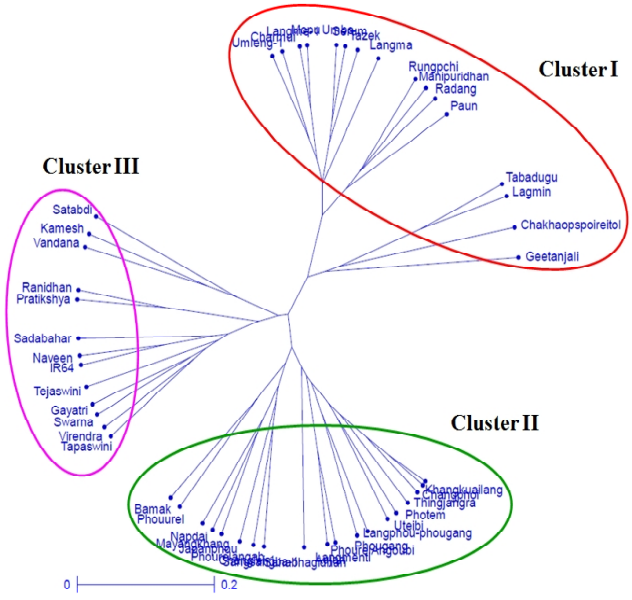

Fig. 1. UPGMA tree showing the genetic relationship among 48 genotypes based on 33 cold stress linked SSR markers.

Table 1. List of 48 indica rice lines used in the present study and their response to seedling stage chilling stress tolerance.

Sl.No.	Name of genotype	Response to chilling stress	Sl.No.	Name of genotype	Response to chilling stress
1	Geetanjali	Very Highly Tolerant	25	Photem	Moderately Tolerant
2	Sahabhagidhan	highly Susceptible	26	Napdai	Moderately Susceptible
3	Satabdi	Moderately Tolerant	27	Changphoi	Moderately Tolerant
4	Kamesh	Highly Tolerant	28	Aujari	Moderately Tolerant
5	Vandana	Moderately Tolerant	29	Khangkuailang	Moderately Tolerant
6	Paun	Highly Tolerant	30	Mayangkhang	Moderately Tolerant
7	Radang	Highly Tolerant	31	Langmenti	Moderately Tolerant
8	Serum	Highly Tolerant	32	Japanphou	Moderately Tolerant
9	Manipuridhan	Highly Tolerant	33	Gayatri	Moderately Susceptible
10	Rungpchi	Highly Tolerant	34	Naveen	Moderately Susceptible
11	Langphou-phougang	Moderately Tolerant	35	IR64	Moderately Tolerant
12	Charmui	Highly Tolerant	36	Phouurel	Moderately Susceptible
13	Umleng-1	Very Highly Tolerant	37	Chakhaopspoireitol	Moderately Tolerant
14	Tazek	Highly Tolerant	38	Ranidhan	Moderately Susceptible
15	Mopu	Highly Tolerant	39	Tejaswini	Moderately Susceptible
16	Lagmin	Highly Tolerant	40	Virendra	Moderately Tolerant
17	Umbo	Highly Tolerant	41	Swarna	Moderately Susceptible
18	Langme-1	Highly Tolerant	42	Tapaswini	Moderately Susceptible
19	Bamak	Highly Tolerant	43	Phourelangab	Moderately Tolerant
20	Changlei-1	Moderately Susceptible	44	Pratikshya	Moderately Susceptible
21	Sangsangba-1	Moderately Tolerant	45	Langma	Very Highly Tolerant
22	Uteibi	Moderately Tolerant	46	Tabadugu	Tolerant
23	Sadabahar	Moderately Susceptible	47	PhourelAngoubi	Moderately Tolerant
24	Thingjangra	Moderately Tolerant	48	Phougang	Moderately Tolerant

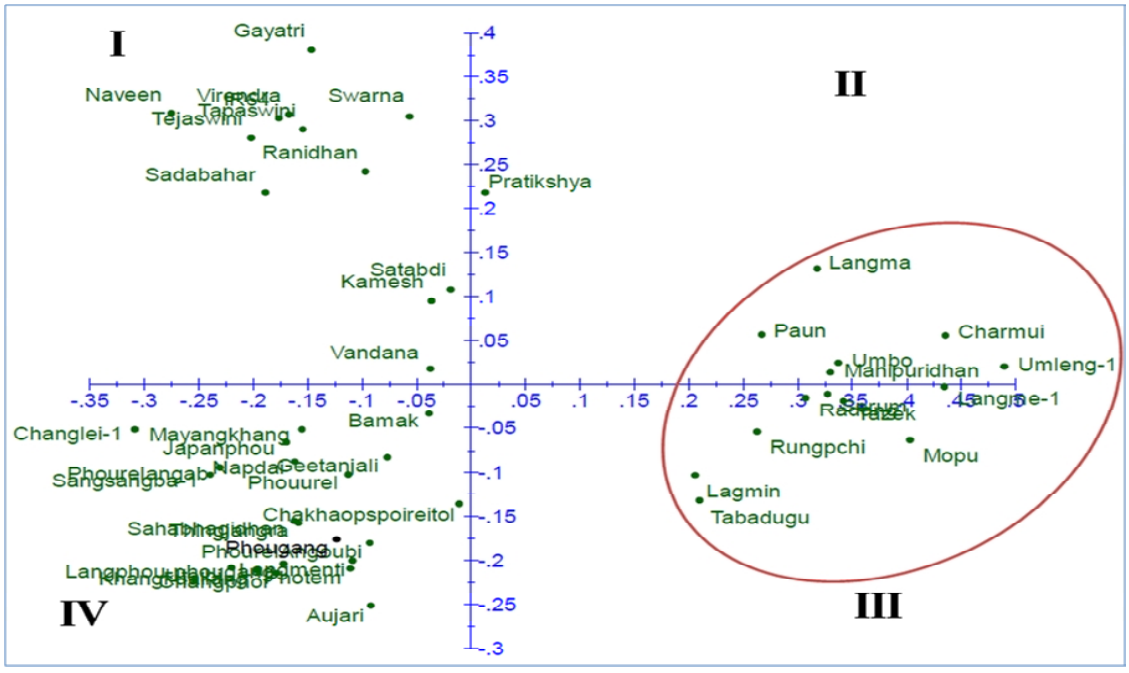

Fig. 2. Principal Coordinate Analysis (PCoA) of panel population based on seedling stage cold tolerance linked 33 SSR markers.

genotypes in the 4th quadrant. The susceptible genotypes were placed in the 1st quadrant (Fig. 2).

Genetic diversity

A panel of 48 genotypes comprising tolerant and susceptible types was genotyped using 33 linked SSR markers for chilling stress tolerance. All the loci used and their genetic diversity parameters obtained are presented in Table 2. *In toto*, 136 amplicons were obtained with 33 markers. An average of 4.1 alleles per locus was detected with a range of 1 to 9 per marker. The PIC value ranged from 0.2067 (RM6651) to 0.7341 (RM297) with an average of 0.5575. The observed average heterozygosity (H_o) was 0.1659 which varied between 0.00 and 0.89. Seven markers showed H_o value to be zero, whereas rest 26 markers showed H_o value higher than zero. The average gene diversity or average heterozygosis (H_e) was 0.6142 ranging between 0.2342 (RM6651) and 0.7712 (RM297). The major allele frequency of these linked polymorphic markers ranged from 0.2979 to 0.8646 with an average of 0.4971 (Table 2).

Population structure

The population panel was analyzed for genetic structure on the Bayesian clustering approach taking probable sub-populations (K) and selecting higher ΔK value, an ad hoc quantity related to the second order change of the log probability of data for the number of clusters detected by Structure (Evano et al., 2005). A high ΔK peak value of 410.18 was observed among the assumed K at K=3 as per the Evano table output (Fig. 3; Table 3). Another peak was also observed at K=5 with ΔK value of 141.87. Hence, both the K values were tested for grouping of the genotypes into different sub-populations. At K=2, the sub-population 1 (SP1) contained 15 genotypes with 13 pure and 2 admixture types, accommodating highly and very highly tolerant genotypes to seedling stage cold tolerance. A total of 33 genotypes present in sub-population 2 (SP2) representing susceptible and moderately tolerant sub-population were with all pure type to the sub-population (Fig. 3; Table 4). Maximum allele frequency divergence between populations observed between the two groups, SP1 and SP2 was 0.1982. The fixation index values (F_{ST}) of the sub-populations were found to be 0.4246 and 0.5863 for SP1 and SP2 respectively. Further, the program exhibited a lower value of alpha (α=0.0589) in the population panel. At K=5, the SP1, SP2, SP3, SP4 and SP5 consisted 12, 10, 6, 10 and 10 no. of genotypes respectively. SP1, SP2, SP4 and SP5 included one admix type each, whereas SP3 included all pure

Table 2. Details of 33 SSR markers used for genotyping the panel indica rice genotypes and their genetic diversity parameters.

Marker	Major allele frequency	No. of alleles detected	Gene Diversity	Heterozygosity	PIC	f
RM3602	0.4167	4.0	0.7081	0.0833	0.6585	0.8846
RM1347	0.4583	5.0	0.6808	0.1042	0.6299	0.8499
RM5746	0.3750	4.0	0.6777	0.0625	0.6115	0.9096
RM5704	0.3750	5.0	0.6834	0.2708	0.6237	0.6103
RM286	0.4792	6.0	0.7086	0.1667	0.6776	0.7691
RM297	0.2979	5.0	0.7712	0.1277	0.7341	0.8377
RM328	0.5263	3.0	0.6053	0.0000	0.5344	1.0000
RM152	0.3542	5.0	0.7424	0.3125	0.6991	0.5860
RM1341	0.5000	4.0	0.6300	0.0625	0.5666	0.9028
RM84	0.3936	5.0	0.7325	0.0426	0.6903	0.9431
RM472	0.6667	3.0	0.4783	0.0000	0.4091	1.0000
RM85	0.3438	4.0	0.7250	0.1458	0.6736	0.8026
RM341	0.4375	3.0	0.6241	0.0000	0.5448	1.0000
RM561	0.6596	3.0	0.4989	0.0000	0.4401	1.0000
RM493	0.4468	5.0	0.6956	0.1702	0.6478	0.7599
RM7003	0.8125	3.0	0.3223	0.0625	0.2989	0.8097
RM14978	0.4583	3.0	0.6398	0.0000	0.5664	1.0000
RM2799	0.5978	2.0	0.4809	0.0652	0.3652	0.8671
RM506	0.4271	4.0	0.6656	0.0208	0.6009	0.9693
RM284	0.4167	3.0	0.6554	0.0000	0.5817	1.0000
RM50	0.3854	4.0	0.7070	0.0417	0.6525	0.9423
RM3648	0.3936	5.0	0.7035	0.8936	0.6533	-0.2603
RM239	0.4583	5.0	0.5838	0.7292	0.4949	-0.2392
RM2634	0.5208	6.0	0.6684	0.2500	0.6334	0.6323
RM1812	0.4091	9.0	0.7567	0.0909	0.7279	0.8824
RM558	0.6875	4.0	0.4844	0.2500	0.4432	0.4919
RM4154	0.5106	5.0	0.6096	0.0426	0.5403	0.9316
RM5312	0.5532	4.0	0.6041	0.0426	0.5463	0.9310
RM3701	0.4583	4.0	0.6799	0.5000	0.6273	0.2744
RM590	0.8542	2.0	0.2491	0.0000	0.2181	1.0000
RM1113	0.5313	2.0	0.4980	0.1042	0.3740	0.7948
RM6651	0.8646	2.0	0.2342	0.2708	0.2067	-0.1463
RM9	0.3333	5.0	0.7632	0.5625	0.7249	0.2728
Mean	0.4971	4.1	0.6142	0.1659	0.5575	0.7348

type genotypes.

Cold tolerance breeding for seedling stage is important in both boro and dry season rice (Pandit et al., 2017). Existence of genetic diversity for the trait is an essential step for developing low temperature stress tolerant high yielding variety that can be cultivated during dry season. The panel population under the present study was diverse in terms of their response to chilling stress including 3 very highly tolerant, 13 highly tolerant, 1 tolerant, 19 moderately tolerant and 12 moderate to highly susceptible genotypes. The molecular diversity study also showed moderate to high genetic diversity with average PIC value more than 0.5 with maximum range of 0.734. Around 74% markers showed PIC value more than 0.5 indicating their ability to classify the genotypes. Moderate to high level of genetic diversity for various traits were earlier reported in rice (Garris et al., 2003; Agrama and Eizenga, 2008; Jin et al., 2010; Chen et al., 2011; Zhang et al., 2011; Zhao et al., 2013; Shah et al., 2013; Singh et al., 2013; Salgotra et al.,

Table 3. Evanno table output showing delta K value to determine the inferred number of sub-populations (K).

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
1	10	-3599.55	0.925263	-	-	-
2	10	-3115	0.514242	484.55	210.93	410.1768
3	10	-2841.38	0.667999	273.62	144.5	216.3178
4	10	-2712.26	7.998639	129.12	7.14	0.892652
5	10	-2576	0.945163	136.26	134.09	141.8697
6	10	-2573.83	215.0266	2.17	126.32	0.587462
7	10	-2445.34	13.34584	128.49	75.56	5.661688
8	10	-2392.41	19.67906	52.93	16.17	0.821686
9	10	-2355.65	22.15713	36.76	30.9	1.394585
10	10	-2287.99	23.46787	67.66	-	-

Table 4. The inferred ancestry value and population structure in the panel population containing 48 genotypes.

Sl No	Name of Genotype	Response to seedling stage cold stress	Inferred ancestry at K=2			Inferred ancestry at K=5					
			Q1	Q2	Structure group	Q1	Q2	Q3	Q4	Q5	Structure group
1	Geetanjali	VHT	0.016	0.984	SP2	0.002	0.006	0.003	0.977	0.011	SP4
2	Sahabhagidhan	HS	0.008	0.992	SP2	0.009	0.618	0.329	0.04	0.003	SP2
3	Satabdi	MT	0.041	0.959	SP2	0.007	0.011	0.031	0.859	0.091	SP4
4	Kamesh	HT	0.07	0.93	SP2	0.004	0.004	0.005	0.943	0.044	SP4
5	Vandana	MT	0.049	0.951	SP2	0.004	0.009	0.003	0.962	0.023	SP4
6	Paun	HT	0.981	0.019	SP1	0.928	0.011	0.005	0.018	0.038	SP1
7	Radang	HT	0.997	0.003	SP1	0.985	0.002	0.001	0.01	0.002	SP1
8	Serum	HT	0.992	0.008	SP1	0.975	0.002	0.002	0.002	0.018	SP1
9	Manipuridhan	HT	0.998	0.002	SP1	0.994	0.002	0.001	0.002	0.001	SP1
10	Rungpchi	HT	0.949	0.051	SP1	0.945	0.02	0.014	0.016	0.004	SP1
11	Langphou-phougang	MT	0.003	0.997	SP2	0.006	0.975	0.003	0.006	0.011	SP2
12	Charmui	HT	0.998	0.002	SP1	0.995	0.001	0.001	0.002	0.001	SP1
13	Umleng-1	VHT	0.999	0.001	SP1	0.996	0.001	0.001	0.001	0.001	SP1
14	Tazek	HT	0.984	0.016	SP1	0.956	0.008	0.006	0.028	0.003	SP1
15	Mopu	HT	0.997	0.003	SP1	0.994	0.002	0.002	0.002	0.001	SP1
16	Lagmin	HT	0.602	0.398	SP1	0.043	0.002	0.002	0.952	0.001	SP4
17	Umbo	HT	0.994	0.006	SP1	0.988	0.005	0.002	0.003	0.002	SP1
18	Langme-1	HT	0.998	0.002	SP1	0.994	0.001	0.001	0.001	0.002	SP1
19	Bamak	HT	0.06	0.94	SP1	0.013	0.003	0.477	0.503	0.004	SP4
20	Changlei-1	MS	0.002	0.998	SP2	0.001	0.002	0.994	0.001	0.002	SP3
21	Sangsangba-1	MT	0.002	0.998	SP2	0.001	0.045	0.947	0.004	0.003	SP3
22	Uteibi	MT	0.003	0.997	SP2	0.002	0.98	0.004	0.01	0.004	SP2
23	Sadabahar	MS	0.003	0.997	SP2	0.004	0.005	0.039	0.005	0.946	SP5
24	Thingjangra	MT	0.015	0.985	SP2	0.008	0.98	0.002	0.008	0.002	SP2
25	Photem	MT	0.019	0.981	SP2	0.007	0.986	0.002	0.004	0.001	SP2
26	Napdai	MS	0.004	0.996	SP2	0.002	0.003	0.985	0.008	0.002	SP3
27	Changphoi	MT	0.004	0.996	SP2	0.001	0.994	0.002	0.001	0.001	SP2
28	Aujari	MT	0.012	0.988	SP2	0.002	0.975	0.017	0.004	0.003	SP2
29	Khangkuailang	MT	0.004	0.996	SP2	0.001	0.993	0.003	0.002	0.001	SP2
30	Mayangkhang	MT	0.005	0.995	SP2	0.003	0.003	0.986	0.005	0.004	SP3
31	Langmenti	MT	0.01	0.99	SP2	0.004	0.944	0.033	0.003	0.016	SP2
32	Japanphou	MT	0.004	0.996	SP2	0.005	0.028	0.96	0.002	0.004	SP3
33	Gayatri	MS	0.005	0.995	SP2	0.002	0.001	0.002	0.001	0.994	SP5
34	Naveen	MS	0.002	0.998	SP2	0.001	0.003	0.002	0.002	0.993	SP5
35	IR64	MT	0.003	0.997	SP2	0.003	0.003	0.003	0.003	0.99	SP5
36	Phouurel	MS	0.005	0.995	SP2	0.002	0.035	0.126	0.833	0.004	SP4
37	Chakhaopspoireitol	MT	0.032	0.968	SP2	0.003	0.104	0.008	0.874	0.011	SP4
38	Ranidhan	MS	0.021	0.979	SP2	0.014	0.033	0.007	0.038	0.908	SP5
39	Tejaswini	MS	0.006	0.994	SP2	0.002	0.002	0.038	0.003	0.955	SP5
40	Virendra	MT	0.004	0.996	SP2	0.001	0.002	0.002	0.005	0.99	SP5
41	Swarna	MS	0.031	0.969	SP2	0.012	0.002	0.003	0.002	0.98	SP5
42	Tapaswini	MS	0.005	0.995	SP2	0.001	0.009	0.004	0.007	0.979	SP5
43	Phourelangab	MT	0.008	0.992	SP2	0.005	0.006	0.932	0.056	0.002	SP3
44	Pratikshya	MS	0.075	0.925	SP2	0.009	0.003	0.002	0.362	0.624	SP5
45	Langma	VHT	0.994	0.006	SP1	0.967	0.002	0.002	0.022	0.007	SP1
46	Tabadugu	T	0.512	0.488	SP1	0.031	0.003	0.002	0.963	0.001	SP4
47	PhourelAngoubi	MT	0.053	0.947	SP2	0.007	0.482	0.024	0.485	0.002	SP4
48	Phougang	MT	0.007	0.993	SP2	0.005	0.728	0.248	0.015	0.004	SP2

VHT: Very highly tolerant, HT: Highly tolerant, T: Tolerant, MT: Moderately Tolerant, MS: Moderately Susceptible, HS: Highly susceptible.

2015). The PCoA and UPGMA cluster analysis using 33 chilling stress linked molecular markers could differentiate the genotypes according to their response to the cold stress. The tolerant, moderately tolerant and

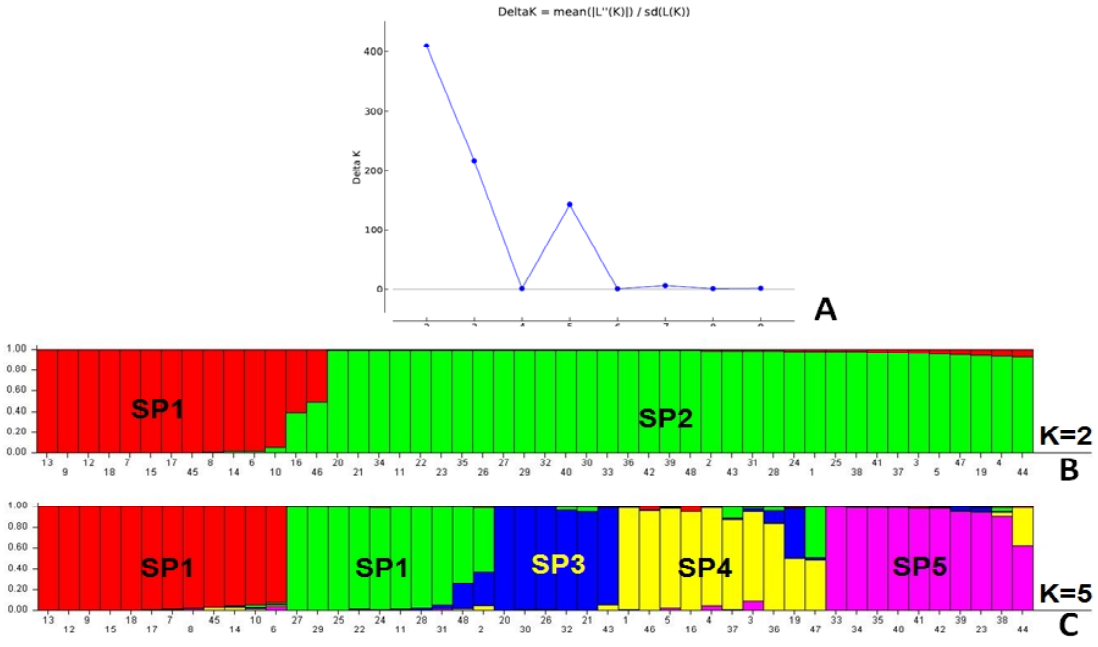

Fig. 3. (A) Graph of estimated membership probability fraction for K = 2 and K = 5. The maximum of adhoc measure ?K determined by structure harvester was found to be K = 2 with a another peak at K=5, (B) Population structure of a panel based inferred ancestry at K = 2 and (C) Population structure of a panel based inferred ancestry at K = 5.

susceptible ones were clearly grouped into different clusters/quadrants (Fig. 1 & 2). This indicates the effectiveness of these markers to distinguish the tolerant genotypes from moderately tolerant and susceptible ones. The QTLs associated with these markers are effectively expressing in these genotypes and can be used for pyramiding to other high yielding backgrounds for developing high yielding chilling stress tolerant genotypes. Different sub-groups obtained with these linked markers suggested that there might be existence of many genes/QTLs for chilling stress tolerance in the selected population. This also suggested that expression of different gene(s)/QTL(s) and their possible combinations in this panel might be responsible for the level of tolerance of these genotypes for seedling stage chilling stress tolerance. As this study indicated presence of multiple QTLs for the trait, it was important to study the population structure of the panel population for the trait. The panel population could be grouped into two sub-populations separating the tolerant genotypes from the susceptible ones. A lower value of alpha (α=0.0589) was detected from which it can be inferred that in most of the landraces, the trait had a common primary ancestor. Similar opinions were also reported in previous publications (Mather et al., 2004; Zhao et al., 2013; Pradhan et al., 2016, Pandit et al., 2017). Very high FST values obtained for SP1 (0.4246) and SP2 (0.5863) suggested higher genetic variation among the genotypes in the sub groups. The significant FST among the clusters indicate a real variation in these clusters (Pandit et al., 2017).

In the present study, moderate to high level of genetic diversity for seedling stage chilling tolerance was noticed by using the panel population. The population structure analysis revealed entire population could be into two sub-populations differentiating the tolerant lines from the susceptible ones. Further, it could be concluded that most of the genotypes had a common primary ancestor for the trait. The donor lines in the panel exhibited the presence of different QTLs for the expression of tolerance for cold stress. Hence, these tolerant genotypes can be used as donor lines in breeding programs. The linked molecular markers that could clearly classify the highly tolerant, moderately tolerant and susceptible genotypes will be useful for marker-assisted breeding programs to develop cold tolerant lines.

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