Delineation of Navara Punja - a Unique Germplasm in *Oryza sativa* cv. Navara Complex in Kerala, India using Morphological and SSR Markers

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The diversity analysis among the indigenous medicinal rice germplasm of Navara (*Oryza sativa* L. cv. Navara) using morphological, biochemical and molecular parameters showed genetic polymorphism in this landrace. Germplasm characterization has been attempted using 35 qualitative and 48 quantitative traits and it revealed distinct variation for 6 vegetative and 10 reproductive qualitative traits. Analysis of variance for 48 quantitative traits showed significant variation ($P<0.05$) for six traits within accessions viz. endosperm elongation ratio, number of leaves (40^{th} day), leaf width, flag leaf width, panicle length and spikelet distance. Cluster analysis using qualitative and quantitative traits grouped Navara punja as a separate cluster. Protein (40.7mg/g) and total free amino acid content (2.06mg/g) was significantly high in accessions of Navara punja when compared to other ecotypes of Navara landraces and released varieties. High degree of polymorphism was exhibited by 27 SSR markers among Navara races. Unique bands of molecular weight 207.2bp were amplified from RM20 which delineated Navara punja from other Navara races. Results showed existence of a unique genotype in Navara germplasm namely Navara punja apart from Navara golden yellow and Navara black.

Key Words: Morphological evaluation, Navara germplasm, Navara punja, Nutritional traits, SSR

Introduction

Many of the Indian rice varieties have known medicinal properties which have been traditionally used in Ayurveda, Unani and by traditional healers for generations. India depends on this crop as a common man food and is also considered sacred in religious ceremonies in temples. In Kerala, the southernmost state in India, among the indigenous germplasm for medicinal rices, Navara is important as it is traditionally cultivated for ayurvedic medicine due to its properties in alleviating rheumatism and neural disorders (Sreejayan *et al*., 2005). As per GI (Geographical Indication) registry, 2008, registration for this rice landrace was obtained as "Navara" by the Navara rice farmer's society, Palakkad, Kerala and hence the epithet "Navara" (Yasodharan, 2008) is used in describing the landrace.

As most farmers are shifting to other cultivars of rice, availability of Navara is diminishing and the germplasm is becoming endangered. It is therefore necessary to collect, preserve and characterize Navara for increasing awareness among the people on the importance of this landrace.

Traditionally Navara rice has been classified as black and golden yellow on the basis of its glume color (Leena Kumary, 2004). Sreejayan *et al.* (2003) classified Navara into 4 ecotypes *viz*. long yellow (Kuttanadan), short yellow (Palakkadan), intermediate yellow (Vadakkan) and short black (Wynadan). Apart from these landraces, Navara punja is a rare medicinal landrace found only in certain parts of northern Kerala especially Kannur and Kasaragod districts. Local farmers of this area consider this landrace as more superior to other Navara races.

Studies including morphological evaluation and SSR markers were carried out to characterize and analyze the genetic relationship of Navara germplasm with other traditional rice landraces (Mariet *et al.,* 2010). Though many attempts have been made to study the Navara germplasm in different perspectives (Menon and Potty, 1997, 1999, 2005; Sreejayan *et al.,* 2003, 2005; Jiji *et al.,* 2007*;* Sanal Kumar *et al.,* 2008, 2010*)* the existence of Navara punja, a unique race, in this complex has not been revealed till date. In this background, a biosystematic approach in studying the germplasm using morphological, biochemical and molecular traits has been undertaken to characterize and delineate Navara punja as a separate entity in Navara germplasm complex.

Materials and Methods

Germplasm of Navara along with three released cultivars

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viz. PTB-35 (Annapurna; PTB-20 X IR8), PTB-50 (Kanchana; Pavizham (HS) X IR36) and PTB-39 (Jyothi: PTB-10 X IR8) was procured from different agroecosystems of Northern Kerala. Locations were mapped using GPS and the germplasm was evaluated under organic farming in Randomized Block Design in triplicates for five consecutive seasons. In each season, data of five plants selected at random from each replicate was recorded and the average data from each season was analyzed. Germplasm characterization has been attempted using 35 qualitative and 48 quantitative traits following Standard Evaluation System for rice (IRRI, 2002). Qualitative and quantitative traits were classified into 155 and 121 sub traits respectively and their presence or absence was scored as 1 or 0. Data analysis has been carried out using NTSYS pc version-2.02i (Numerical Taxonomy and multivariate System) and SPSS-13.0 for qualitative and quantitative traits respectively. Analysis of variance (One-way ANOVA) was performed for 48 quantitative traits using SPSS 13.0. Biochemical analysis for primary metabolites viz. protein (Lowry *et al.,* 1951), total free amino acids (Lee and Takahashi, 1966), starch and total carbohydrates (Hedge and Hofreiter, 1962) were carried out to find variability in nutritional traits among Navara germplasm.

Young leaves of 20-day-old seedlings of various Navara races were used for genomic DNA isolation following the CTAB method (Doyle and Doyle, 1990). SSR analysis was carried out in order to determine the genetic uniqueness among different ecotypes of Navara complex. Twenty seven SSR markers (Ocimum Biosolutions, USA) were used for SSR analysis to find level of polymorphism among landraces.

Touchdown PCR (Applied Biosystems, USA) was performed for SSR analysis. PCR amplification profile has initial denaturation step of 95ºC for 5min. It was followed by ten cycles of 94ºC for 30 sec, 65ºC for 30 sec and 72ºC for 30sec with 10ºC decrease in temperature per cycle. This was followed by 30 cycles of 94ºC for 30 sec, 56ºC for 30 sec and 72ºC for 60 sec. Final extension was carried out at 72ºC for 20 min and was held at 4ºC. Amplified DNA fragments were subjected to electrophoresis with marker DNA of known molecular wt. (100-500bp) in 2.8% agarose gel at 6V/cm using 1X TAE buffer and Ethidium Bromide (0.5 µg/ml) was used for staining. Gel was viewed and photographed using gel documentation system (Syngene G-Box, USA).Data analysis was carried out using NTSYS-pc version 2.02i and Jaccard's similarity co-efficient generated were used in constructing dendrogram with UPGMA (Unweighted Pair Group of Arithmetic averages).

Results

The characterization and evaluation of the Navara germplasm at morphological, biochemical and molecular levels presented distinct variations and the results showed the delineation of Navara punja as a separate entity in Navara germplasm complex.

Locations from where the germplasm has been procured have been marked using GPS and the passport information is given in Table 1.

Morphological Characterization

Characterization of the germplasm for 35 qualitative traits revealed distinct variation for 6 vegetative and 10 reproductive traits. Erect leaves, partly exerted panicle,

Table 1. Passport information of germplasm analyzed

Sl. No.	IC Number/ other ID	Cultivar name	Regions explored	District	State	Latitude	Longitude	Altitude
1	539968	Navara black	Chitur	Palakkad	Kerala	10º41.534'	76º43.186'	289ft
2	539983	Navara golden yellow	Kolazhi	Thrissur	Kerala	10º59.319'	76º54.785'	642ft
3	86475	Navara black with awn	NBPGR regional station	Thrissur	Kerala	10º59.319'	76º54.785'	642ft
4	539998	Navara golden yellow	Thrikkaripur	Kasaragod	Kerala	12º08.055'	75º10.094'	274ft
5	557561	Navara punja	Morazha	Kannur	Kerala	11º59.259'	75º21.140'	152ft
6	557562	Navara punja	Morazha	Kannur	Kerala	11º59.259'	75º21.140'	152ft
7	557564	Navara punja	Kanul	Kannur	Kerala	11º59.620'	75º21.377'	160ft
8	557587	Navara black	Maruthur	Palakkad	Kerala	12º03.275'	75º15.693'	206ft
9	557588	Navara golden yellow	Malakakkattil	Palakkad	Kerala	12º03.275'	75º15.693'	206ft
10	PTB 35	Annapurna	Pattambi	Palakkad	Kerala	12º03.275'	75º15.693'	206ft
11	PTB 50	Kanchana	Chittur	Palakkad	Kerala	10º41.591'	76º43.275'	361ft
12	PTB 39	Jyothi	Pattambi	Palakkad	Kerala	10º41.591'	76º43.275'	361ft

golden yellow glume and light brown seed coat are the traits which showed variation in Navara punja (IC557561, IC557562, and IC557564). Negligible variations were also observed in collar color, blade color, apiculous color, internode color, coleoptile color, early plant vigor and root hairiness. Cluster analysis using UPGMA generated dendrogram with one base cluster dividing the germplasm into three clusters at 63% similarity (Fig.1). Jaccard's co-efficient of similarity ranged between 0.52 and 0.92. Navara black (IC539968 and IC557587) formed first cluster showing a similarity of 70%, whereas second cluster had all the released varieties (PTB35, PTB50 and PTB39). All the Navara punja accessions viz. IC557561, IC557562, and IC557564 formed third cluster. IC557561 and IC557564 presented a similarity of 90%, but IC557562 had only 82% similarity with these two accessions. Navara punja ecotypes showed 62% similarity with Navara black (IC539968 and IC557587), whereas it showed 70% similarity with IC539983 (Navara golden yellow) and 67.5% with IC557588 (Navara golden yellow). Genotype IC86475 (Navara black with awn) clustered separately at 52% and had the least similarity co-efficient with other cultivars.

Dendrogram generated based on 121 sub-traits of 48 quantitative characters revealed 2 major clusters at a similarity coefficient of 0.50 (Fig. 2). Jaccard's co-efficient of similarity ranged between 0.37 and 0.83. Cluster I was subdivided into two sub clusters and the first sub cluster included two accessions of Navara black (IC539968 and IC557587). Navara golden yellow (IC539983 and IC557588) and Navara punja (IC557561, IC557562, and IC557564) formed second sub cluster. Second major cluster grouped released varieties (PTB-35, PTB-50, PTB-39) and Navara black with awn (IC86475). In first sub cluster, IC539968 presented a similarity of 67% with IC557587 and the similarity coefficient of IC539983 with IC557588 was 61%. Navara punja showed 50% similarity with Navara black, Navara black with awn and released varieties. But similarity coefficient of Navara punja with Navara golden yellow was 56%. Among Navara punja accessions, similarity coefficient of IC557561 and IC557562 was 83% and that of IC557564 with other two accessions was 70%. Genotype IC86475 (Navara black with awn) presented a similarity coefficient of 50% with other cultivars.

Analysis of variance (One-way ANOVA) for 48 quantitative traits has been carried out and significant variation ($P<0.05$) was recorded for six traits within accessions viz. endosperm elongation ratio, number of leaves (40^{th} day), leaf width, flag leaf width, panicle length and spikelet distance. Among Navara cultivars, Navara punja showed maximum mean values for

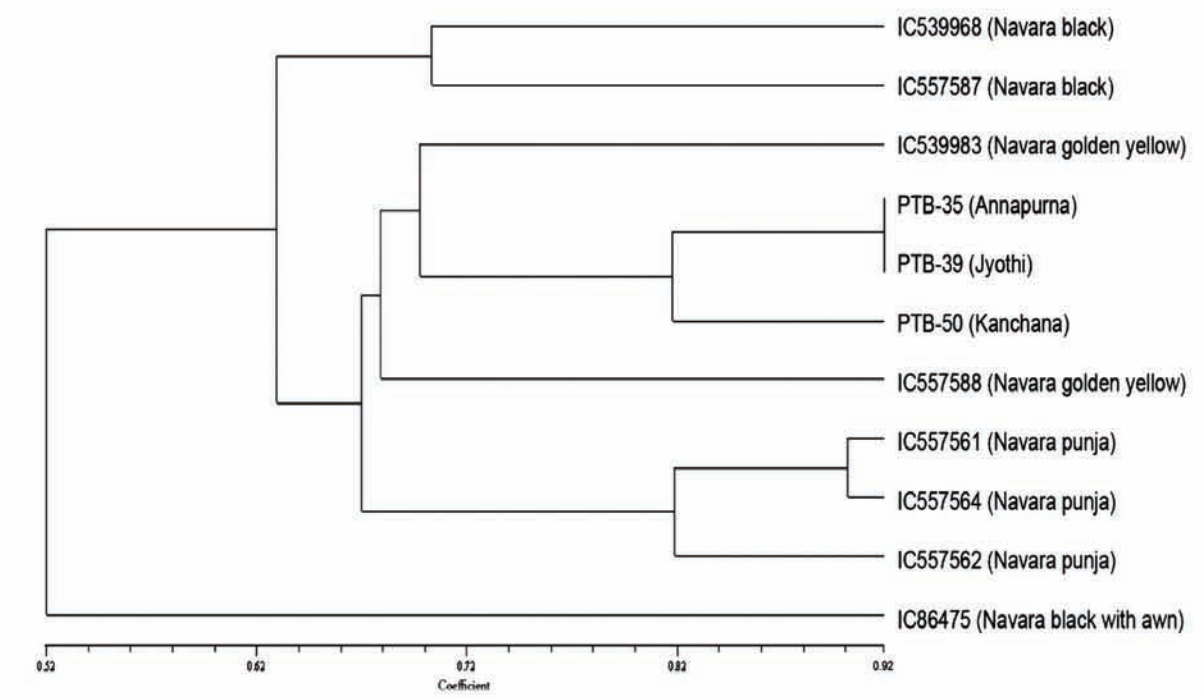

Fig. 1. Dendrogram generated from qualitative traits

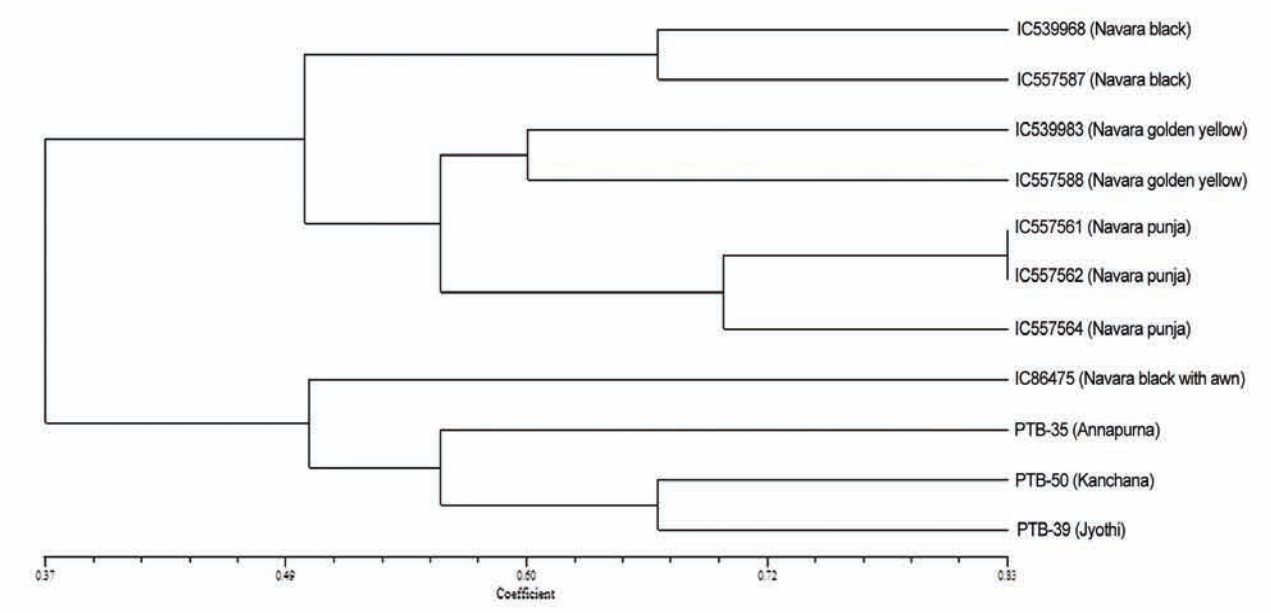

Fig. 2. Dendrogram generated from quantitative traits

elongation ratio, widening ratio, flag leaf length, ligule length, culm diameter, days to 50% flowering, days to 100% flowering, days to maturity and panicle length. These traits also showed significant variation among these cultivars (Table 2).

Quantification of Primary Metabolites

Estimation of primary metabolites viz. protein, total free amino acids, total carbohydrate and starch among different Navara accessions also showed distinct variation (Table 2). Protein (40.7mg/g) and total free amino acid content (2.06mg/g) was significantly high in accessions of Navara punja when compared to other ecotypes of Navara landraces and released varieties. Relative increase of total carbohydrate and starch content (742.35mg/g and 187.14mg/g respectively) was observed in released varieties than medicinal rice, Navara.

SSR Analysis

Genetic diversity across 11 rice genotypes was analyzed using twenty seven SSR markers. RM 28050, RM 257, RM 337, RM 168 and RM 20 grouped Navara punja as a distinct landrace. RM 20 (Chr.12, p) was the only SSR marker displaying unique bands (Mwt: 207.2bp) for all the three Navara punja accessions (IC557561, IC557562 & IC557564*)* (Fig. 3). In Navara punja accessions, SSR markers RM 208, RM 210, RM 190, RM 225, RM 518 and RM 212 generated bands for IC557561, whereas RM 282 produced unique bands for IC557562 and RM 535, RM 6100, RM 611, RM 167 for IC557564. Two hundred and forty eight bands were generated from 27 SSR markers and average number of bands obtained per marker was 9.92. Markers exhibited a polymorphism of 63.36% (Table 3). Diversity among genotypes was assessed using UPGMA analysis.

In the present study, SSR analysis showed Navara landrace and released rice varieties as genetically different. Dendrogram generated based on SSR markers revealed 2 major clusters as given in Figure 4. Each cluster was sub divided into two clusters at similarity co-efficient of 0.11. Jaccard's co-efficient of similarity varied from 0.03 to 0.40. In cluster I, sub clusters comprised of Navara black (IC539968 and IC557587) and released varieties (PTB-39 and PTB-50). Similarity coefficient of Navara punja, IC557562 and IC557564 was 20% and that of IC557561 with other two accessions was 19%. Genetic distance was found to be least for IC 86475(Navara black with awn) and IC557588 (Navara golden yellow). Thus SSR markers helped in revealing genetic diversity within three ecotypes of Navara landrace.

Discussion and Conclusion

Distinct variation observed in morphological traits viz. erect leaves, partly exerted panicle, golden yellow glume and light brown seed coat can be used as markers in identifying Navara punja among other ecotypes of Navara. Similar studies for identifying Navara genotypes have been reported by Sanal Kumar *et al.* (2010).

Table 2. Variability in quantitative traits and primary metabolites

Serial No	Traits	Navara black				Navara golden yellow			Navara punja				Released varieties				Standard error	Significant variation
		IC539968	IC86475	IC557587	Average	IC539983	IC557588	Average	IC557561	IC557562	IC557564	Average	PTB-35	PTB-50	PTB-39	Average		
1	Seed length(mm)	7.85	7.32	7.75	7.64	7.5	6.5	7.02	6.61	6.62	7.02	6.75	8.07	7.58	6.87	7.51	.03523	.000
2	Seed breadth(mm)	1.52	1.85	1.77	1.71	1.51	1.58	1.55	1.62	1.46	1.61	1.56	1.59	1.77	1.77	1.71	.03523	.264
3	Seed Length-breadth ratio (mm)	5.22	4.23	4.53	4.65	4.87	4.14	4.51	4.11	4.54	3.81	4.15	5.12	4.32	4.03	4.49	.12414	.250
4	Endosperm length before boiling (mm)	7.08	6.31	6.44	6.61	6.16	6.4	6.28	6	5.92	6.34	6.08	7.02	6.44	5.97	6.48	.06176	.000
5	Endosperm length after boiling (mm)	8.18	7.38	7.32	7.62	7.12	6.78	6.95	6.98	7.08	7.36	7.14	7.36	7.08	7.02	7.15	.06452	.000
6	Elongation ratio (mm)	1.15	1.17	1.14	1.15	1.16	1.06	1.11	1.17	1.19	1.16	1.17	1.05	1.11	1.18	1.11	.01129	.047
7	Endosperm width before boiling (mm)	1.33	1.5	1.32	1.38	1.27	1.38	1.33	1.32	1.08	1.3	1.23	1.28	1.5	1.42	1.4	.03285	.315
8	Endosperm width after boiling (mm)	1.68	1.9	1.8	1.79	1.64	1.75	1.69	1.7	1.52	1.7	1.64	1.74	1.78	1.83	1.78	.03018	.334
9	Widening ratio (mm)	1.27	1.26	1.36	1.3	1.29	1.26	1.27	1.28	1.41	1.31	1.33	1.36	1.18	1.30	1.28	.01823	.312
10	Sterile lemma length (mm)	7.88	7.39	7.76	7.67	7.7	6.6	7.18	6.69	6.62	7.04	6.78	8.1	7.64	6.68	7.47	.09348	.000
11	Sterile palea length (mm)	7.34	7.02	7.32	7.22	7.34	6.18	6.76	6.32	6.32	6.54	6.39	7.53	7.32	7.68	7.16	.08340	.000
12	Percentage germination	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	00000	0.00
13	Day of completion of germination	4.4	4.2	4.4	4.3	4.6	4.2	4.4	4.4	4.4	4.4	4.4	4	4.2	4.4	4.2	.06703	.966
14	Germination speed	23	24	23	23.3	24	24	24	23	23	23	23	25	24	23	24	.06703	.917
15	Radicle length (cm)	2.92	3.95	2.44	3.13	1.49	2.62	2.06	1.56	2.36	1.53	1.82	3.39	3.91	4.85	4.05	.16322	.000
16	Plumule length (cm)	6.53	5.09	3.01	4.87	1.13	3.27	2.2	2.49	2.48	1.85	2.27	4.4	3.87	5.91	4.73	.26216	.000
17	Vigor Index	652.6	508.6	300.6	487.3	176	326.6	251.3	248.6	247.8	184.6	227	440.4	387.2	591.2	472.9	25.9883	.000
18	1st leaf length (cm)	2.93	3.69	2.45	3.02	2.0	2.74	2.37	3.12	3.65	2.98	3.25	2.78	3.19	3.32	3.09	.12181	.207
19	1st leaf breadth (cm)	0.21	0.22	0.19	0.21	0.19	0.17	0.18	0.27	0.29	0.18	0.24	0.21	0.28	0.29	0.26	.00774	.000
20	Height upto 1st leaf (cm)	4.99	4.72	7.56	5.75	2.73	4.69	3.71	5.29	6.09	5.23	5.53	4.43	4.53	3.54	4.17	.22536	.000
21	No. of leaves on 40th day	3.79	3.75	4.05	3.86	3.63	3.92	3.78	4.09	3.96	4.03	4.03	4.05	4.03	3.95	4.01	.03326	.088
22	Seedling ht on 40th day (cm)	29.95	31.5	32.51	31.32	32.96	31.84	32.37	25.8	23.51	28.72	26.01	18.5	16.9	26	20.47	.99332	.307
23	Leaf length (cm)	31.95	38.15	28.31	32.8	30.26	33.64	31.95	36.8	36.51	30.72	34.68	33.52	26.9	28.89	29.77	1.11928	.513
24	Leaf width (cm)	0.49	0.87	0.53	0.63	0.64	0.62	0.63	0.73	0.65	0.59	0.66	0.8	0.76	0.66	0.74	.02400	.387
25	Culm No on 60th day	4.06	7.45	3.79	4.27	4.93	4.36	4.65	5.05	4.49	4.98	4.84	4.39	4.96	4.86	4.74	.24359	.156
26	No of tiller on 60th day	3.13	6.39	2.72	4.08	3.8	2.69	3.24	4.09	3.26	3.99	3.78	2.97	3.95	3.81	3.57	.24270	.070
27	No. of effective tiller on 60th day	2.44	2.77	2.48	2.56	2.87	2.39	2.63	2.95	2.68	2.59	2.74	2.6	3.23	2.57	2.8	.15421	.877

Table 2. continued

Serial No	Traits	Navara black				Navara golden yellow			Navara punja				Released varieties				Standard error	Significant variation
		IC539968	IC86475	IC557587	Average	IC539983	IC557588	Average	IC557561	IC557562	IC557564	Average	PTB-35	PTB-50	PTB-39	Average		
27	No. of effective tiller on 60th day	2.44	2.77	2.48	2.56	2.87	2.39	2.63	2.95	2.68	2.59	2.74	2.6	3.23	2.57	2.8	.15421	.877
28	Culm length before flowering (cm)	32	33.4	41.98	35.77	44.14	40.35	42.25	29.97	27.27	34.55	30.59	21.11	20.91	19.65	20.56	1.83319	.080
29	Flag leaf length (cm)	23.42	28.69	23.49	25.2	25.98	26.39	26.19	29.06	28.70	24.66	27.47	24.92	23.51	17.76	22.06	.90083	.125
30	Flag leaf width (cm)	0.68	0.92	0.57	0.72	0.74	0.61	0.66	0.81	0.73	0.64	0.73	1.02	0.85	0.88	0.92	.02858	.020
31	Ligule length (cm)	0.62	0.88	1.02	0.84	1.08	1.07	1.07	1.09	1.49	1.21	1.26	1.33	0.6	1.14	1.12	.06786	.041
32	Culm diameter (mm)	2.52	3.65	1.86	2.68	1.38	3.01	2.2	2.84	2.61	2.74	2.72	2.31	2.88	4.28	3.16	.18311	.061
33	Days to heading	79	90	61	77	71	67	69	77	81	78	79	97	98	97	97	1.46689	.076
34	Days to flowering	83	94	65	81	75	72	74	82	85	83	83	103	105	103	104	1.46144	.054
35	Days to 50% flowering	87	97	67	84	79	75	77	87	89	90	89	110	112	109	110	1.53711	.012
36	Days to 100% flowering	92	104	76	91	85	82	84	92	96	95	94	121	122	121	121	1.62607	.020
37	Days to maturity	122	131	116	123	115	123	119	127	131	128	129	151	154	150	152	1.25837	.006
38	Panicle length (cm)	13.4	14.6	13.8	13.9	14.1	11.3	12.7	19.9	18.5	15.8	18.1	13.74	15.2	15.5	14.8	.51981	.007
39	No. of primary branches	4.15	5.19	4.38	4.57	4.36	4.3	4.33	5.03	5.2	4.98	5.07	3.39	6.03	3.98	4.46	.10343	.000
40	No .of Panicle	3.74	3.52	4.69	3.98	2.62	3	2.81	3.45	3.80	4.03	3.76	2.36	2.95	2.44	2.58	.11120	.000
41	Spikelet distance(cm)	0.56	0.57	0.51	0.55	0.53	0.53	0.53	0.59	0.57	0.50	0.55	0.56	0.47	0.50	0.51	.00811	.067
42	Total seeds per/panicle	47.2	63.34	56.21	55.58	41.14	50.59	45.86	58.46	60.12	56.64	58.4	63.52	63.41	62.72	63.2	3.15487	.711
43	Sterile seeds per/panicle	22.61	25.87	24.67	24.38	16.48	18.27	17.38	21.04	20.65	17.65	19.78	28.72	25.41	25.32	26.48	1.53357	.912
44	% panicle sterility	48.4	40.72	42.54	43.88	41.01	35.01	38.01	40.8	36.43	37.24	38.15	45.42	43.76	43.29	44.16	2.34273	.802
45	Fertile seeds/per panicle	24.59	37.47	31.54	31.2	26.66	32.33	29.5	37.42	39.47	38.99	38.62	34.8	37.99	37.4	36.73	2.63302	.859
46	% panicle fertility	51.54	59.24	57.45	56.08	59.08	54.88	56.98	59.2	63.57	62.73	61.83	54.57	56.23	56.7	55.8	2.20597	.880
47	Single plant yield (g)	8.08	8.96	8.05	8.36	9.15	8.02	8.58	9.85	9.24	10.17	9.75	8.52	7.22	9.36	8.36	.13410	.000
48	Hundred seed weight (g)	1.52	2.02	1.51	1.68	1.33	1.44	1.39	1.55	1.27	1.39	1.4	1.61	1.71	1.77	1.7	.03125	.000
	Biochemical analysis																	
1	Protein(mg/g)	29.66	23.61	37.24	30.17	39.1	38.05	38.57	41.31	40.84	39.82	40.65	28.	26.	27.51	27.6	.73313	.996
2	Total free amino Acids(mg/g)	1.83	1.05	0.64	1.17	0.88	1.95	1.41	2.06	2.07	2.06	2.06	1.8	0.36	0.52	0.89	.08258	1.000
3	Starch(mg/g)	149.45	145.35	70.13	121.6	102.59	128.33	115.46	123.59	92.22	145.59	120.4	187.44	162.83	211.16	187.44	5.08784	1.000
4	Total carbohydrates (mg/g)	657.63	564.57	338.58	520.26	471.67	544.77	508.22	500.46	538.71	647.13	562.1	665.45	777.23	784.37	742.35	15.81776	.998

Table 3. SSR markers and bands obtained for Navara Punja

SSR Marker	Chromosome	Position	Total No. of Amplicons	No. of Polymorphic bands	% polymorphism	IC number	Molecular weight of band	Rf value
RM 212	Chr.1	Q(148.7cm)	10	8	80	IC557561	189.29	0.345
RM 208	Chr.2	Q(186.4cm)	9	4	44.44	IC557561	299.9	0.429
RM 535		Q(195.7cm)	10	7	70	IC557564	114.13	0.576
RM 282	Chr.3	P(100.6cm)	9	7	77.77	IC557562	145.95	0.614
RM 168		Q(171.2cm)	11	6	54.54	IC557562 IC557564	93.61 84.79	0.504 0.516
RM 518	Chr.4	P(25.5cm)	11	7	63.63	IC557561	156.68	0.768
RM 317		Q(118.3cm)	10	7	70	IC557561 IC557562	163.41 68.29	0.479 0.474
RM 611	Chr.5	P	5	5	100	IC557564	546.6 131.25	0.264 0.442
RM 225	Chr.6	P(26.2cm)	10	7	70	IC557561	124.99	0.625
RM 190		Q(7.4cm)	9	5	55.55	IC557561	105.64	0.630
RM 337	Chr.8	P(1.1cm)	14	11	78.57	IC557561 IC557564	181.4, 151.16 62.79	0.452, 0.482 0.470
RM 210		Q(90.3cm)	6	5	83.33	IC557561	163.41	0.544
RM 201	Chr.9	Q (81.2cm)	11	6	54.54	IC 557561 IC557564	104.69 84.78	0.332 0.340
RM 257		Q(66.1cm)	11	8	72.72	IC557561 IC557564	122.22 05.56	0.548 0.562
RM 6100	Chr.10	Q	11	5	45.45	IC557564	139.62	0.574
RM 167	Chr.11	P(37.5cm)	10	7	70	IC557564	70.31	0.449
RM 20	Chr.12	P(3.2cm)	11	7	63.63	IC557561 IC557562 IC557564	207.2	0.529
RM 28050		P	9	7	77.77	IC557561 IC557564	163.48 51.58	0.430 0.442

Quantitative traits also showed distinct variation in Navara punja apart from other Navara landraces. Navara punja showed variation in flag leaf width, ligule length, culm diameter and panicle length from other cultivars. Days to 50% flowering, days to 100% flowering and days to maturity are also more in Navara Punja than other two Navara races.

Cluster analysis of qualitative and quantitative morphological traits revealed the existence of three forms of Navara viz. Navara black, Navara golden yellow and Navara punja. Dendrogram generated by cluster analysis revealed Navara punja as a separate entity. The similarity coefficient within accessions of Navara punja also showed variation revealing phenotypic variability even within this ecotype. Thus qualitative and quantitative analysis of morphological data delineates Navara punja from other Navara ecotypes.

Quantification of primary metabolites revealed that Navara Punja has high protein and amino acid content compared to other Navara landraces and released varieties. Navara punja and other Navara landraces were also characterized by low starch and total carbohydrate content compared to released varieties. Maximum amount of free amino nitrogen and incorporation of amino acids, in rice grains with high protein content was reported by Lourdes *et al* (1970).

Analysis of SSR profiles showed similarity in dendrogram generated from morphological traits. Navara germplasm was found to be genetically different from released varieties as the genetic distance between them

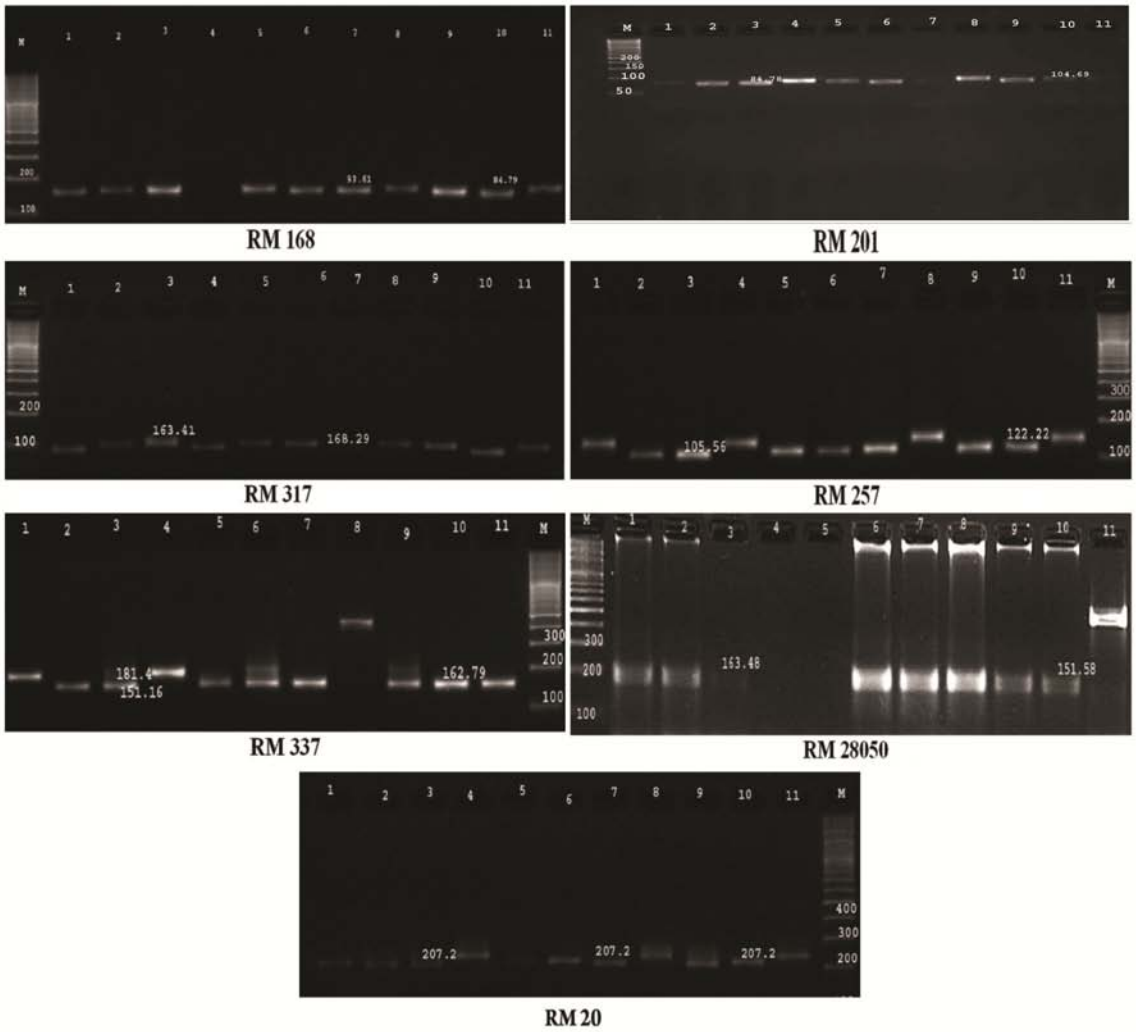

Fig. 3. SSR profile for differentiating *Oryza sativa* cv. Navara punja.Lane 1-Navara black (IC539968); Lane 2-Navara golden yellow (IC539983); Lane 3-Navara punja (IC557561); Lane 4-Annapurna (PTB-35); Lane 5-Navara black with awn (IC86475); Lane 6-Navara golden yellow (IC557588); Lane 7-Navara punja (IC557562); Lane 8-Kanchana (PTB-50); Lane 9-Navara black (IC557587); Lane 10-Navara punja (IC557564) and Lane 11-Jyothi (PTB-39)

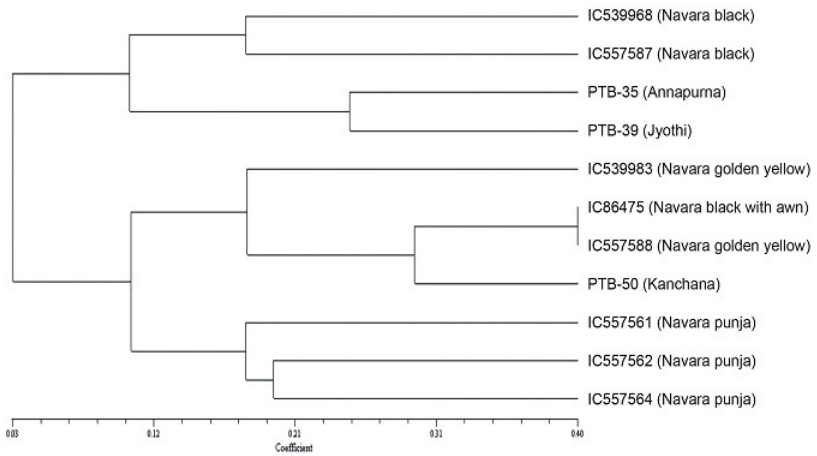

Fig. 4. Dendrogram generated based on SSR markers

was high. Allelic frequencies of Navara landraces also showed clustering of Navara landraces into three different entities. The unique bands exhibited by RM 20 for the three accessions of Navara punja suggest the utilization of this marker for the characterization of Navara punja. Similarity coefficient within Navara punja accessions was observed to be varying. SSR markers viz. RM 208, RM 210, RM 190, RM 225, RM 518 and RM 212 was found to be specific to IC557561, whereas RM 282 was specific to IC557562, and RM 535, RM 6100, RM 611, RM 167 for IC557564. Thus it reveals that genetic variability exist even within Navara punja. Similar work has been carried out by Sreejayan *et al.* (2005) in Navara landraces using molecular markers *viz.* SSR where Navara was clustered into three different morpho types and varieties forming a distinct group. The unique bands observed for Navara punja accessions and clustering of Navara punja into a separate group confirm delineation of Navara punja as a distinct morphotype among the Navara land race.

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