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Study on Apparent Amylose Content in Context of Polymorphism Information Content along with Indices of Genetic Relationship Derived through SSR Markers in *Birain*, *Bora* and *Chokuwa* Groups of Traditional Glutinous Rice (*Oryza sativa* L.) of Assam

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Abstract: Amylose content was determined in 41 traditional glutinous rice varieties of Assam classed as *Birain*, *Bora* and *Chokuwa* group during 2004-06. Average apparent amylose content in 6 accessions of *Chokuwa* (9.368%) was higher than 20 accessions of *Bora* group (0.502%) and 15 accessions of *Birain* (0.191%) genotypes. *Mahsuri,* a non-glutinous rice variety contained intermediate amylose content (21.2%). Eight SSR markers were used to assess genetic variability. The size of amplified fragments ranged from 100 to 500 bp. Among all genotypes, average Polymorphism Information Content (PIC) was 0.923. The average genetic similarity within the *Birain* accessions ranged from 0.119 to 0.571. Within *Bora-Chokuwa* accessions, similarity value ranged from 0.047 to 0.667. The average similarity was 0.228, which reflected that the *Bora* group could be more diverse than the *Birain* group. Amylose content is said to be highly influenced by environmental conditions. Since, *Birain* accessions were from the same Barak valley agro-climatic condition and *Bora* as well as *Chokuwa* were from the Brahmaputra valley, an analysis was made with corresponding pair-wise relative rate of increase (%) in apparent amylose contents as well as corresponding values of pair-wise Jaccard's co-efficient of similarity among the accessions of *Birain*, *Bora* and *Chokuwa* groups of glutinous rice. It showed the existence of a matching relation between the increased values of respective apparent amylose content and the genetic similarity. It seems that apparent amylose content though cannot play a solid indicator for genetic variability in glutinous rice germplasm. However it may help to gauge biochemical bases towards genetic variability under same environmental condition.

Key words: Starch, Waxy rice, SSR markers, coefficient of similarity

INTRODUCTION

Glutinous rice is also called sticky rice, sweet rice, Waxy rice, *Botan* rice, *Mochi* rice and pearl rice. It is a type of short-grained rice that is especially sticky when cooked and is grown in Japan, Korea, China, Philippines, Thailand, Indonesia, India and Vietnam. This class of rice was introduced to Assam from Thailand or Burma a considerable time ago (Sharma *et al*., 1971). The Waxy rice of Assam has been classified in two groups, *Bora* (glutinous) and *Chokuwa* (semi-glutinous) based on apparent amylose content. *Birain* or *Beruin* rice is also another class of glutinous rice popular in Barak valley in the southern region of Assam, India and adjoining areas of Bangladesh. It is called glutinous in the sense of being glue-like or sticky and not in the sense of containing gluten. Its sticky properties should not be confused with the other varieties of rice that become sticky to one degree or another when cooked. Waxy rice of Assam has significance in social and religious ceremonies and forms a

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popular daily breakfast diet in rural Assam. Milled rice is also used in the preparation of snacks, flat rice, puffed rice, bamboo rice, sweet rice beer and other dishes. The parboiled polished *Chokuwa* rice swells on soaking and becomes soft and is used by the people of Assam as a fast food with curd. These multiplicities of uses make the glutinous rice very popular among farmers, in spite of the advent of modern high yielding rice varieties. What distinguishes glutinous rice from other types of rice is having no (or negligible amounts of) amylose content. Amylose, a linear polymer of glucose is one of two components of starch, the other being amylopectin. The apparent amylose content of *Chokuwa* varieties varies from 15 to 20%, while that of *Bora* varieties was found in trace quantities (Dutta and Barua, 1978).

To date more than 2000 SSR markers of cultivated rice are available, which provides a powerful tool for studying *Oryza* genus as a whole, as SSR markers have good cross-species amplification (McCouch *et al*., 2002). Waxy locus has been evaluated with SSR markers. Ayres *et al*. (1997) determined the relation between the Wx microsatellite polymorphism and the amylose content in the 92 US rice strains. They demonstrated that the Wx microsatellite is polymorphic enough to distinguish most rice strains in different amylose classes and the inheritance of the CT repeats can be traced through a cross-section of the US rice pedigree. Bergman *et al*. (2001) demonstrated that the CT repeats classes of Wx gene with n = 10 or 11, 14, 20 and 17 and 18 were categorized as high amylose, intermediate amylose and low-amylose types, respectively. Bao (2002) reported the usefulness of (CT)n microsatellitic markers of Wx gene in rice starch quality improvement. Prathepha (2003) characterized 68 rice strains belonging to two species of *Oryza* by using Wx microsatellite, (CT)n repeats, that are closely linked to the rice waxy gene. Prathepha (2003) observed the predominance of (CT)17 repeats in the Wx locus, in addition to identification of a unique microsatellite class,(CT)18 repeats, in the strains tested. While evaluating amylose content or identifying Waxy locus, no study has been reported to link amylose content with indices of genetic relationship among the glutinous germplasm for appropriate characterization. The present investigation was carried out to study variation for apparent amylose content in context of polymorphism information content along with indices of genetic relationship derived through SSR markers in *Birain*, *Bora* and *Chokuwa* groups of traditional glutinous rice of Assam.

MATERIALS AND METHODS

Seeds of 41 glutinous rice varieties (Table 1) were used in the present study during 2004-06. Among them, 15 (suffixing the word *Birain*) were collected from Regional Agricultural Research Station (RARS), Assam Agricultural University (AAU), Karimganj and the remaining 26 (suffixing the words *Bora* and *Chakuwa*) were from RARS, AAU, Titabor, representing distinct agro-climatic variation within the plains of the two river valleys, the former one from the Barak valley and rest two from the Brahmaputra valley of the northeast India. The traditional names as given by the farmers were retained in the germplasm accessions for field level identification. Karnel of *Pusha Birain*, *Das Birain*, *Mow Birain*, *Kala Birain*, *Agirjal Birain*, *Akib Birain*, *Gela Bora*, *Memon Bora*, *Chokuwa Bora-2* and *Parochokuwa Sali* were red while rests were white. The accessions like *Uba Birain*, *Gela Bora*, *Jaisungam Birain*, *Das Birain*, *Maju Chokuwa* and *Pakhiloga Bora* could be classed as slender grain and rest were as either non-slender or bold.

Amylose content was determined by the method described by Sawbhagya and Bhattacharya (1979). Defatted moisture-free powdered (100 mg) sample was taken in a stoppered conical flask. One milliliter of distilled alcohol was added to wet the powder. Ten milliliter of 1 N NaOH was then gently added by the side and kept for overnight and heated next day in a boiling water bath for 2-3 min. After cooling, the volume was made up to 100 mL with distilled water. Five milliliter of above dispersion was transferred into a 100 mL volumetric flask. Fifty milliliter of water was added to it, followed by

Table 1: Apparent amylose content in (AACP) and corresponding pair-wise relative rate of increase (%) in AACP as well as corresponding values of pair-wise Jaccard's co-efficient of similarity within *Birain*, *Bora* and *Chokuwa* groups of glutinous rice accessions of Assam

Glutinous rice accessions in *Birain* group	AACP	Corresponding pair-wise relative rate of increase in AACP (%)	Value of corresponding pair-wise Jaccard's coefficient of similarity	Glutinous accessions rice in *Bora* group	AACP	Corresponding pair-wise relative rate of increase in AACP (%)	Value of corresponding pair-wise Jaccard's coefficient of similarity
Jaisungam Birain	0.136			*Joha Bora*	0.151		
Chefa Birain	0.146 →	7.35 →	0.300	*Kaun Bora*	0.155 →	2.65 →	0.064
Pani Birain	0.155 →	6.16 →	0.238	*Rupohi Bora*	0.163 →	5.16 →	0.365
Tepra Birain	0.163 →	5.16 →	0.244	*Memon Bora*	0.163 →	0.00 →	0.286
Agirjal Birain	0.165 →	1.23 →	0.289	*Boga Bora*	0.176 →	7.98 →	0.333
AkibBirain	0.172 →	4.24 →	0.400	*Rangali Bora*	0.178 →	1.12 →	0.362
Mow Birain	0.189 →	9.88 →	0.119	*Tangun Bora*	0.185 →	3.78 →	0.353
Jhanki Birain	0.189 →	0.00→	0.325	*Pakhiloga Bora*	0.185 →	0.00 →	0.204
Kacha Birain	0.195 →	3.17 →	0.275	*Til Bora*	0.189 →	2.16 →	0.242
Kala Birain	0.195 →	0.00 →	0.306	*Chokuwa Bora-2*	0.191 →	1.06 →	0.156
Das Birain	0.200 →	3.33 →	0.308	*Ranga Bora-3*	0.208 →	8.90 →	0.179
Aki Birain	0.219 →	9.50 →	0.212	*Gela Bora*	0.219 →	5.30 →	0.065
Garuchakhuki Birain	0.246 →	12.33 →	0.222	*Bor Bora*	0.219 →	0.00 →	0.326
Uba Birain	0.248 →	0.81 →	0.313	*Chandra Bora*	0.355 →	62.1 →	0.389
Pusha Birain	0.250 →	0.81 →	0.324	*Ranga Bora-1*	0.468 →	1.83 →	0.208
***Chokuwa* group**				*Jengoni Bora*	0.470→	0.43 →	0.119
Parochokuwa Sali	2.400			*Garuchakuwa Bora*	0.678 →	44.25 →	0.378
Sam Chokuwa	5.360 →	123.33 →	0.129	*Bora-2*	0.680 →	0.30 →	0.235
Maju Chokuwa	7.840 →	46.27 →	0.268	*Ghew Bora*	0.708 →	4.12 →	0.242
Bor Chokuwa	11.04 →	40.82 →	0.280	*Chokuwa Bora-1*	2.400 →	238.98 →	0.190
Boga Chokuwa	11.20 →	1.45 →	0.304	**Non-glutinous check**			
Kalamdani Chokuwa	11.40 →	1.79 →	0.317	*Mahsuri*	21.200		

→ Value corresponding to glutinous rice accession in the previous row

the addition of 1 mL of acetic acid and 2 mL of iodine (0.2%) solution. The volume was made up to the mark with distilled water; pH of this solution was maintained at 4.5. One milliliter of standard amylose solution was taken and treated using the same procedure. A reagent blank was also prepared as such except for adding amylose extract or the standard amylose solution. After 30 min of interval, the intensity of colour developed was measured in spectrophotometer (Systronics UV-VIS, Model-118) at 630 nm against the reagent blank. Amylose content was calculated using the following relationship:

$$\text{Amoylose content} = \frac{R}{A}\times\frac{a}{r}\times\frac{1}{5}\times 100$$

Where:
R = 630 nm reading for sample dispersion
A = 630 nm reading for standard amylose solution
a = Amount of the standard amylose taken
r = Amount of sample taken

The estimation was done in triplicate and their mean was recorded as g of amylose per 100 g in moisture free sample.

For studying genetic variation through SSR techniques, the total genomic DNA from each of the genotypes was extracted following the protocol of Plaschke *et al.* (1995) with slight modification. Genetic relatedness among the genotypes was then computed by using the Jaccard's coefficient of similarity using SIMQUAL module of NISYS-pc. The pair-wise genetic similarity index was calculated as per Jaccard's coefficient of similarity (Jaccard, 1908) is given below:

$$F = NAB1 / (NT - NAB0)$$

where, F is similarity index; NAB1 is number of bands present (Scored 1) in both accessions A and B; NAB0 is number of bands present in all test entries but not present in accession A or B; NT is total number of bands scored in the study.

RESULTS AND DISCUSSION

Apparent Amylose Content

The *Birain* genotypes had the lowest amylose content (0.191%) followed by bora genotypes (0.502%) and *Chokuwa* genotypes (9.368%), respectively. Among the glutinous rice accessions, the maximum and minimum amylose containing genotypes were *Kalamdani Chokuwa* (11.400%) and *Jaisungam Birain* (0.136%), respectively. Among *Birain* genotypes, the maximum and minimum amylose containing genotypes were *Pusha Birain* (0.250%) and *Jaisungam Birain* (0.136%), respectively. Among *Chokuwa* genotypes, the maximum amylose containing genotype was *Kalamdani Chokuwa* (11.400%) and the minimum amylose containing genotype was *Sam Chokuwa* (5.360%). Considering *Bora* genotypes alone, the maximum and minimum amylose containing genotypes were *Ghew Bora* (0.708%) and *Joha Bora* (0.151%), respectively. Present investigation revealed that the amylose content of indigenous glutinous rice ranged from 0.136 to 11.4%, indicating some of them as sticky or Waxy rice. In general, *Birain* group (0.191%) has lower amylose content than *Bora* genotypes (0.502%). Based on amylose content, Juliano *et al.* (1981) classified rice as Waxy (0-2% amylose), very low (5-12%), low (12-20%), intermediate ((20-25%) or high (25-30%). Dutta and Baruah (1978) reported amylose content in *Bora* genotypes as trace amount and that in *Chokuwa* from 17.14 to 19%, which were more than that observed in the present study. But Kandali *et al.* (1995) reported 0.95 to 1.25% of amylose in *Bora* accessions of Assam, which are in close correspondence with that observed in the present investigation. There is a possibility of duplication in Assam rice collection by having the similarity in names. It has been observed that the accessions with similar names (two *Chokuwa Bora* accessions and two *Ranga Bora* accessions) had different level of amylose content (Table 1). Moreover, amylose content is highly influenced by environmental conditions (Juliano and Pascual, 1980). So, based on amylose content alone, the duplicate nature of the four accessions could not be ascertained, warranting the use of better system for analyzing genetic variability and duplicate identification. However, for *Birain* group of Barak valley, no published report on amylose content is available. Glutinous rice lacks the starch amylose, which constitutes up to 30% of the total starch in non-glutinous rice endosperm (Oka, 1988). The glutinous phenotype arises through the disrupted expression of the amylose biosynthesis gene, (Wx), which encodes a granule-bound starch synthase (Sano, 1984). Glutinous rice contains a G to T mutation at the 5′splice site of Wx intron 1 that leads to incomplete post-transcriptional processing of Wx pre-mRNA (Wang *et al*., 1995; Bligh *et al*., 1998; Cai *et al*., 1998; Hirano *et al*., 1998; Isshiki *et al*., 1998). Glutinous rice does not have detectable levels of spliced mRNA as a result of this mutation (Wang *et al*., 1995; Bligh *et al*., 1998). But some degree of amylose synthesis is restored in varieties that carry the mutation due to display of cryptic splice site activation (Cai *et al*., 1998; Olsen and Puragganan, 2002). This might be the reason for detecting very low level of amylose in the present study and other similar studies in glutinous rice of Assam. Dipti *et al*. (2003) reported that the amylose content of *Beruin* rice of Bangladesh ranged from 7.9 to 10%, which was much higher than that obtained in *Birain* rice of Assam (0.136 to 0.250%) from the present study. Such differences in amylose content might be due to differences in genotypes under investigation along with environmental variation. However, methods to estimate amylose content might influence the results, which cannot be confirmed from the present investigation. *Mahsuri*, a non-glutinous rice variety contained intermediate amylose content (21.2%) remains soft and fluffy on cooking. Vanaja and Babu (2006) reported that *Mahsuri* contains 23.64% amylose, which is slightly more than the present study.

Therefore, it seems that the apparent amylose content in *Chokuwa* (9.368%) was higher than *Bora* (0.502%) and *Birain* (0.191%) genotypes.

Polymorphism Information Content (PIC)

Eight SSR markers were used in the study to access genetic variability. The size of amplified fragments ranged from 100 to 500 bp. Average Polymorphism Information Content (PIC) was 0.923 in all the genotypes. The marker RM-11 showed a maximum PIC (0.954) and RM-164 showed a minimum PIC (0.876) across all the genotypes (Table 2). Within *Bora-Chokuwa* group, RM-11 generated maximum PIC (0.949) and RM-251 revealed a minimum PIC of 0.837. Within *Birain* accessions, RM-251 revealed a maximum PIC (0.981) and RM-315 showed a minimum PIC of 0.906 (Table 3). A lower PIC was observed for *Bora-Chokuwa* genotypes (0.903) than that for *Birain* genotypes (0.947).

Indices of Genetic Relationship

In all the genotypes, the genetic similarity ranged from 0.029 to 0.667 with an average similarity of 0.238. Maximum similarity (0.667) was observed between *Parochokuwa Sali* and *Til Bora*, while the minimum similarity (0.029) was exhibited by *Tepra Birain* and *Joha Bora* (Table 4).

Within the *Birain* accessions, similarity value ranged from 0.119 to 0.571 with an average of 0.306. The highest similarity (0.571) was exhibited by *Chefa Birain* with *Aki Birain* and *Kala Birain*, whereas, the lowest similarity value (0.119) was observed between *Mow Birain* and *Akib Birain*. Within *Bora-Chokuwa* accessions, similarity value ranged from 0.047 to 0.667. The accessions. *Parochokuwa Sali* and *Til Bora* exhibited maximum similarity (0.667) and *Joha Bora* and *Maju Chokuwa* showed minimum similarity (0.047). The average similarity was 0.228, which reflected that *Bora* group is more diverse than the *Birain* group.

Table 2: Level of polymorphism in few glutinous rice germplasm of Assam as detected by SSR markers

Primers	Range of frequencies of amplified fragments	Average frequency	PIC (Polymorphic Information Content)	Size of most frequent band (bp)
RM-315	0.122-0.512	0.251	0.936	140
RM-282	0.098-0.561	0.268	0.928	150
RM-11	0.049-0.512	0.213	0.954	100
RM-241	0.146-0.488	0.254	0.935	100
RM-251	0.244-0.439	0.305	0.906	140
RM-164	0.195-0.488	0.352	0.876	300
RM-206	0.122-0.415	0.252	0.936	130
RM-224	0.146-0.512	0.282	0.920	100
Average		0.272	0.923	

Table 3: Comparative analysis of level of polymorphism as detected by SSR markers in few glutinous rice germplasm of Assam

	Bora-Chokuwa group				*Birain* group			
Primers	Range of frequencies of amplified fragments	Average frequency	PIC (Polymorphic Information Content)	Size of most frequent alleles (bp)	Range of frequencies of amplified fragments	Average frequency	PIC (Polymorphic Information Content)	Size of most band frequent (bp)
RM-315	0.154-0.462	0.265	0.925	140	0.067-0.800	0.306	0.906	150,140
RM-282	0.077-0.769	0.292	0.914	170	0.067-0.867	0.227	0.948	140,120
RM-11	0.038-0.538	0.225	0.949	170	0.067-0.933	0.282	0.920	150
RM-241	0.115-0.500	0.242	0.941	100	0.067-0.873	0.276	0.924	200
RM-251	0.308-0.538	0.403	0.837	140	0.067-0.533	0.136	0.981	140
RM-164	0.115-0.577	0.290	0.915	300	0.067-0.667	0.207	0.957	350,300
RM-206	0.231-0.654	0.386	0.850	170,160	0.067-0.867	0.193	0.962	130
RM-224	0.154-0.731	0.317	0.899	400,170	0.067-0.733	0.144	0.979	100
Average		0.302	0.903			0.221	0.947	

Table 4: Pair-wise Jaccar's coeffcient of smilarity based on SSR data in glutinous rise accessions under study

Accession name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Aki_Birain	0.571																			
Kacha_Birain	0.323	0.385																		
Pusha_Birain	0.286	0.212	0.200																	
Das_Birain	0.184	0.212	0.263	0.444																
Mow_Birain	0.229	0.188	0.278	0.282	0.351															
Kala_Birain	0.571	0.393	0.306	0.342	0.308	0.256														
Tepra_Birain	0.235	0.194	0.406	0.324	0.441	0.270	0.263													
Pani_Birain	0.238	0.205	0.222	0.311	0.372	0.295	0.415	0.244												
Jhanki_Birain	0.371	0.344	0.275	0.250	0.341	0.325	0.459	0.268	0.409											
Agirjal_Birain	0.216	0.290	0.200	0.300	0.486	0.250	0.378	0.289	0.475	0.375										
Akib_Birain	0.313	0.321	0.216	0.324	0.289	0.119	0.412	0.211	0.302	0.300	0.400									
Jaisungam_Birain	0.300	0.259	0.400	0.179	0.243	0.257	0.324	0.303	0.233	0.361	0.179	0.194								
Uba_Birain	0.556	0.370	0.324	0.324	0.289	0.237	0.500	0.353	0.217	0.300	0.225	0.243	0.265							
Garuchakhuki_Birain	0.267	0.222	0.242	0.364	0.552	0.194	0.294	0.448	0.268	0.231	0.364	0.273	0.182	0.313						
Bor_Bora	0.275	0.150	0.200	0.318	0.289	0.191	0.326	0.222	0.300	0.386	0.234	0.375	0.182	0.279	0.275					
Gela_Bora	0.257	0.182	0.306	0.214	0.244	0.167	0.220	0.231	0.289	0.286	0.214	0.263	0.184	0.263	0.158	0.326				
Memon_Bora	0.263	0.162	0.214	0.310	0.279	0.178	0.317	0.238	0.292	0.289	0.279	0.333	0.195	0.333	0.297	0.564	0.317			
Joha_Bora	0.143	0.125	0.167	0.114	0.147	0.156	0.152	0.029	0.211	0.135	0.147	0.161	0.222	0.125	0.143	0.154	0.152	0.235		
Jengoni_Bora	0.216	0.176	0.200	0.209	0.209	0.111	0.186	0.195	0.255	0.279	0.182	0.324	0.122	0.195	0.154	0.381	0.457	0.222	0.114	
Bora_2	0.243	0.108	0.167	0.262	0.262	0.133	0.300	0.190	0.333	0.273	0.233	0.250	0.237	0.250	0.179	0.341	0.486	0.514	0.250	0.325
Chokuwa_Bora_2	0.242	0.200	0.222	0.143	0.231	0.211	0.237	0.250	0.222	0.378	0.263	0.184	0.167	0.250	0.139	0.174	0.205	0.109	0.094	0.231
Ranga_Bora_3	0.194	0.152	0.150	0.250	0.190	0.297	0.167	0.205	0.326	0.205	0.163	0.119	0.189	0.146	0.162	0.217	0.065	0.178	0.233	0.163
Ranga_Bora_1	0.333	0.207	0.265	0.306	0.382	0.250	0.278	0.257	0.200	0.220	0.205	0.189	0.323	0.294	0.333	0.178	0.179	0.190	0.133	0.119
Boga_Bora	0.170	0.163	0.182	0.327	0.302	0.218	0.214	0.158	0.333	0.358	0.302	0.245	0.212	0.200	0.192	0.389	0.236	0.333	0.191	0.255
Bor_Chokuwa	0.186	0.122	0.174	0.234	0.234	0.167	0.140	0.222	0.226	0.326	0.137	0.196	0.238	0.196	0.159	0.333	0.213	0.298	0.125	0.318
Chokuwa_Bora_1	0.122	0.136	0.234	0.170	0.216	0.224	0.196	0.157	0.327	0.226	0.319	0.180	0.217	0.135	0.122	0.259	0.245	0.226	0.140	0.265
Kalamdani_Chokuwa	0.250	0.143	0.231	0.300	0.333	0.351	0.275	0.324	0.283	0.222	0.300	0.256	0.179	0.225	0.216	0.208	0.275	0.196	0.083	0.182
Boga_Chokuwa	0.175	0.105	0.111	0.174	0.227	0.156	0.152	0.244	0.245	0.239	0.125	0.308	0.200	0.159	0.146	0.250	0.293	0.239	0.051	0.286
Chandra_Bora	0.292	0.239	0.275	0.302	0.278	0.288	0.388	0.269	0.357	0.309	0.278	0.222	0.235	0.375	0.170	0.389	0.360	0.358	0.143	0.327
Maju_Chokuwa	0.214	0.179	0.256	0.208	0.208	0.167	0.239	0.250	0.250	0.298	0.160	0.196	0.268	0.170	0.275	0.255	0.163	0.271	0.047	0.184
Rangali_Bora	0.146	0.111	0.208	0.265	0.319	0.176	0.220	0.180	0.302	0.275	0.216	0.255	0.167	0.204	0.196	0.333	0.196	0.354	0.195	0.319
Kaun_Bora	0.191	0.133	0.204	0.235	0.212	0.245	0.216	0.200	0.321	0.269	0.189	0.224	0.188	0.154	0.120	0.255	0.240	0.245	0.064	0.313
Rupohi_Bora	0.395	0.263	0.217	0.333	0.250	0.208	0.341	0.213	0.314	0.313	0.200	0.239	0.227	0.267	0.293	0.269	0.229	0.286	0.237	0.250
Tangun_Bora	0.238	0.205	0.146	0.229	0.229	0.213	0.208	0.143	0.294	0.292	0.229	0.244	0.233	0.191	0.156	0.275	0.184	0.265	0.150	0.229
Garuchokuwa_Bora	0.170	0.163	0.208	0.216	0.240	0.250	0.151	0.180	0.232	0.226	0.216	0.229	0.167	0.180	0.196	0.308	0.245	0.275	0.140	0.378
Pakhiloga_Bora	0.184	0.143	0.231	0.182	0.333	0.250	0.159	0.289	0.255	0.341	0.268	0.167	0.278	0.225	0.216	0.208	0.186	0.279	0.219	0.209
Parochokuwa_Sali	0.214	0.160	0.088	0.139	0.139	0.182	0.111	0.086	0.116	0.158	0.108	0.152	0.129	0.152	0.172	0.205	0.111	0.222	0.400	0.108
Til_Bora	0.259	0.160	0.088	0.139	0.139	0.219	0.111	0.086	0.200	0.189	0.108	0.152	0.129	0.152	0.172	0.205	0.143	0.222	0.400	0.171
Sam_Chokuwa	0.258	0.259	0.077	0.150	0.179	0.128	0.324	0.132	0.233	0.289	0.211	0.229	0.143	0.229	0.147	0.182	0.216	0.289	0.138	0.278
Ghew_Bora	0.138	0.120	0.200	0.081	0.176	0.086	0.182	0.088	0.205	0.194	0.212	0.121	0.308	0.121	0.179	0.179	0.219	0.229	0.421	0.176

Table 4: Continued

Accession name	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Aki_Birain																				
Kacha_Birain																				
Pusha_Birain																				
Das_Birain																				
Mow_Birain																				
Kala_Birain																				
Tepra_Birain																				
Pani_Birain																				
Jhanki_Birain																				
Agirjal_Birain																				
Akib_Birain																				
Jaisungam_Birain																				
Uba_Birain																				
Garuchakhuki_Birain																				
Bor_Bora																				
Gela_Bora																				
Memon_Bora																				
Joha_Bora																				
Jengoni_Bora																				
Bora_2																				
Chokuwa_Bora_2	0.089																			
Ranga_Bora_3	0.159	0.179																		
Ranga_Bora_1	0.200	0.162	0.184																	
Boga_Bora	0.296	0.250	0.288	0.255																
Bor_Chokuwa	0.204	0.286	0.273	0.233	0.389															
Chokuwa_Bora_1	0.313	0.289	0.224	0.140	0.317	0.214														
Kalamdani_Chokuwa	0.262	0.200	0.190	0.237	0.190	0.115	0.240													
Boga_Chokuwa	0.222	0.316	0.182	0.195	0.246	0.304	0.255	0.317												
Chandra_Bora	0.400	0.250	0.241	0.208	0.365	0.293	0.339	0.255	0.224											
Maju_Chokuwa	0.229	0.200	0.167	0.233	0.271	0.280	0.259	0.261	0.364	0.210										
Rangali_Bora	0.340	0.261	0.277	0.239	0.362	0.308	0.333	0.240	0.333	0.386	0.283									
Kaun_Bora	0.280	0.180	0.220	0.234	0.356	0.353	0.259	0.286	0.327	0.333	0.438	0.377								
Rupohi_Bora	0.326	0.191	0.349	0.310	0.351	0.320	0.167	0.277	0.240	0.375	0.347	0.273	0.365							
Tangun_Bora	0.250	0.222	0.267	0.174	0.310	0.300	0.353	0.204	0.326	0.267	0.354	0.353	0.373	0.426						
Garuchokuwa_Bora	0.235	0.208	0.200	0.239	0.411	0.333	0.241	0.240	0.333	0.386	0.388	0.309	0.431	0.273	0.278					
Pakhiloga_Bora	0.262	0.171	0.220	0.270	0.302	0.450	0.170	0.156	0.200	0.232	0.234	0.319	0.340	0.224	0.204	0.292				
Parochokuwa_Sali	0.135	0.121	0.258	0.161	0.184	0.237	0.085	0.139	0.162	0.137	0.119	0.244	0.182	0.361	0.297	0.109	0.242			
Til_Bora	0.135	0.156	0.345	0.200	0.184	0.237	0.085	0.139	0.194	0.115	0.146	0.214	0.156	0.361	0.333	0.186	0.242	0.667		
Sam_Chokuwa	0.382	0.135	0.100	0.171	0.189	0.130	0.217	0.122	0.231	0.313	0.268	0.244	0.213	0.317	0.205	0.217	0.122	0.129	0.129	
Ghew_Bora	0.242	0.161	0.152	0.167	0.188	0.179	0.190	0.111	0.105	0.213	0.122	0.136	0.109	0.231	0.093	0.163	0.212	0.115	0.115	0.133

1: *Chefa_Birain*, 2: *Aki_Birain*, 3: *Kacha_Birain*, 4: *Pusha_Birain*, 5: *Das_Birain*, 6: *Mow_Birain*, 7: *Kala_Birain*, 8: *Tepra_Birain*, 9: *Pani_Birain*, 10: *Jhanki_Birain*, 11: *Agirjal_Birain*, 12: *Akib_Birain*, 13: *Jaisungam_Birain1*, 14: *Uba_Birain*, 15: *Garuchakhuki_Birain*, 16: *Bor_Bora*, 17: *Gela_Bora*, 18: *Memon_Bora*, 19: *Joha_Bora*, 20: *Jengoni_Bora*, 21: *Bora_2*, 22: *Chokuwa_Bora_2*, 23: *Ranga_Bora_3*, 24: *Ranga_Bora_1*, 25: *Boga_Bora*, 26: *Bor_Chokuwa*, 27: *Chokuwa_Bora_1*, 28: *Kalamdani_Chokuwa*, 29: *Boga_Chokuwa*, 30: *Chandra_Bora*, 31: *Maju_Chokuwa*, 32: *Rangali_Bora*, 33: *Kaun_Bora*, 34: *Rupohi_Bora*, 35: *Tangun_Bora*, 36: *Garuchokuwa_Bora*, 37: *Pakhiloga_Bora*, 38: *Parochokuwa_Sali*, 39: *Til_Bora*, 40: *Sam_Chokuwa*,
Cophenetic correlation, r = 0.663

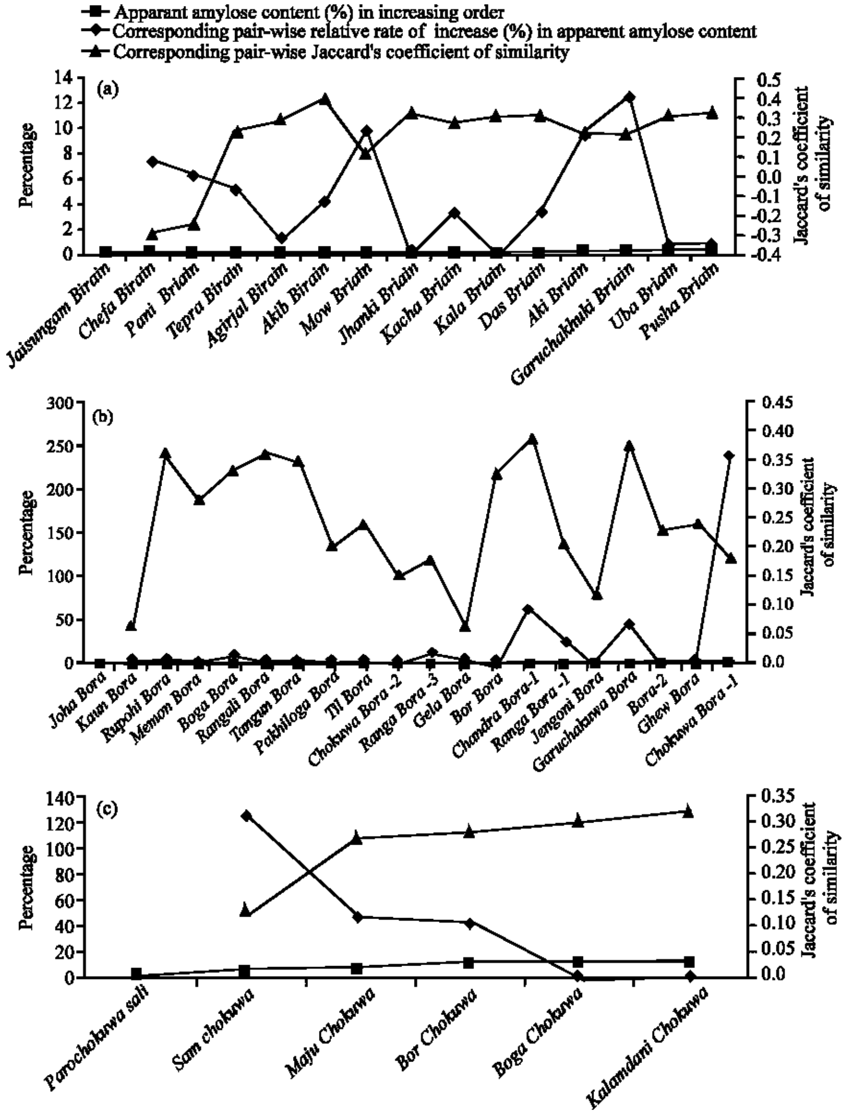

Fig. 1: Apparent amylose content (%) and corresponding pair-wise relative rate of increase (%) in apparent amylose contents as well as corresponding values of pair-wise Jaccard's co-efficient of similarity in (a) *Birain* (b) *Bora* and © *Chokuwa* groups of glutinous rice accessions in Assam

Apparent Amylose Content vis-à-vis Genetic Relationship

Amylose content is said to be highly influenced by environmental conditions. As respective accessions of *Birain*, *Bora* and *Chokuwa* groups were collected from the same environmental condition *viz.* the Barak sub-basin and the Brahmaputra valley agro-climatic conditions, an analysis was therefore made with corresponding pair-wise relative rate of increase (%) in AACP as well as corresponding values of pair-wise Jaccard's co-efficient of similarity among the accessions of *Birain*, *Bora* and *Chokuwa* groups of glutinous rice (Table 1, Fig. 1a-c). It showed the existence of a matching relation between the increased values of respective AACP and the genetic similarity. For instance, in two *Birain* accessions *viz.*, *Akib Birain* and *Mow Birain, Das Birain* and *Garuchakuki Birain,* the AACP was 0.172 and 0.189, 0.200 and 0.246 percent, respectively. Thus the AACP of *Mow Birain*, *Garuchakuki Birain* were about 10 and 23% higher than *Akib Birain and Das Birain,* which in turn, were about 4 and 2.6% higher in amylose content than that of *Agirjal Birain* (0.165) and *Kala Birain* (0.195).

The Jaccard's coefficient (measures similarity) was decreased between *Akib Birain* and *Mow Birain* (0.119) by more than 200% than that of *Akib Birain* and *Agirjal Birain* (0.400) or between *Aki Birain* and *Garuchakuki Birain* (0.222) by more than 39% than that of *Kala Birain* and *Das Birain* (0.308). Similarly, AACP in *Bora* group of accessions, viz., *Ghew Bora* (0.708) was 4% higher than *Bora*-2 (0.680) against its nearest (0.678) *Bora* accession *Goruchokuwa Bora*. The Jaccard's coefficient of similarity between *Goruchokuwa Bora* and *Bora*-2 was 0.378. The genetic similarity between *Ghew Bora and Bora*-2 was decreased by about 61 percent. Thus relative increase in AACP might be a reflection of direct or inverse proportionality to the genetic similarity in *Birain* and *Bora* accessions, which had lower amylose contents. The same reflection was not visualized in *Chokuwa* accessions which had comparatively higher amylose content. Here *Maju Chokuwa* (7.840%) was 46 and 227% higher amylose content than *Sam Chokuwa* (5.360%) and *Parochokuwa* (2.400%), respectively. Genetic similarity between *Sam Chokuwa* and *Maju Chokuwa* (0.268) was however increased by 108% over *Sam Chokuwa* and *Parochokuwa* (0.129). Thus it appeared that the AACP though could not play a concrete indicator for genetic variability in glutinous rice germplasm, however it might help to gauge genetic similarity, especially under lower amylose content. Customarily of course, apparent amylose content may help to characterize geographical indication of Waxy rice germplasm under the existing international patent regime.

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