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Quantification of 2-acetyl-1-pyrroline in joha rice and it's correlation with agro-morphological characters with special reference to upper Brahmaputra valley, Assam, India

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ABSTRACT

Joha rice belongs to aromatic landraces, endemic to Assam and are distinct from other aromatic rice in terms of texture, aroma and cooking quality. 2-Acetyl-1-pyrroline (2AP) is one of the major volatile compounds which contribute to fragrance in aromatic rice. However, the relationship between aroma and low yield have challenged rice breeders to develop other agronomic characters to gain a better yield grain. In this study, 15 joha landraces from different districts of Assam were evaluated for the accumulation of 2AP in the grain along with 52 agro-morphometric characters. Highest 2AP was observed in Dangor Joha (78.667 µg/kg) followed by Kolakunkuni Joha (64.615 µg/kg) and Joha Bora (52.715 µg/kg). Ranikajol Joha showed the lowest accumulation of 2AP in its grain (14.465 µg/kg). Correlation analysis revealed that pubescence of blade surface (r=0.665), width of blade (r=0.57) and density of pubescence of lemma (r=0.509) have positive contribution to 2AP accumulation. Number of panicles per plant have a negative correlation with 2AP (r=-0.26) suggesting that 2AP affects the yield of grain negatively. Therefore, morphological trait such as anthocyanin or colouration in leaf/lemma-palea or high density of pubescence in leaf and lemma would be a potential breeding criterion for selecting joha rice with higher 2AP content.

Figures : 05 References : 21 Table : 01
KEY WORDS : 2-Acetyl-1-pyrroline, Aromatic rice, Correlation, Joha, Landraces, Morphological traits

Introduction

Aromatic rice contributes to a small group among the cultivated rice, but acquires a special reference in context to fragrance or aroma. Aroma is one of the main character or quality which affects and defines the popularity of different food products. Some of the best category of aromatic rice varieties which are internationally recognized includes basmati, jasmine, texmati, tulaipanji, wehani, and wild pecan rice. 2-Acetyl-1-pyrroline (2AP) is one of the important volatile components in aromatic rice and is described to have popcorn-like, roasty, or nutty flavor and sometimes as basmati flavor[3,12]. Apart from aromatic rice varieties, it is biologically synthesized in many other plants including Dadacha-mame varieties of soybeans[5], bread flowers (*Vallaris glabra*)[21] and pandan leaves (*Pandanus amaryllifolius*)[10]. It is also produced by various microorganisms as a secondary metabolite which contributes as an aroma compound to various food products[16].

Joha rice (*Oryza sativa*) are short-grain rice varieties or landraces, which are intermediates of both *indica* and *japonica* variety[13,17]. These varieties are indigenous to Assam and have a characteristic aroma like basmati or jasmine rice, but are different from the later because of their small size, high gluten content, unique aroma, superfine kernel, good cooking qualities and excellent palatability[8,19]. It is not a preferred variety for cultivation due to its low productivity and yield. As a result, many of these varieties have extinct and many are on verge of extinction. Therefore, special strategies should be designed in order to understand the

TABLE-1 : List of Joha rice landraces acquired from the study area and their region of cultivation

S. No.	Landrace	District where cultivation was observed
1	Boga Bhaboli Joha	Sivasagar*
2	Boga Kon Joha	Jorhat*, Sonitpur
3	Boga Kunkuni Joha	Lakhimpur*, Jorhat, Sonitpur, Sivasagar
4	Bos Joha	Jorhat*
5	Dangor Joha	Dhemaji*, Lakhimpur
6	Joha Bora	Sonitpur*
7	Kola Joha 1	Dibrugarh*, Dhemaji, Lakhimpur, Jorhat, Sonitpur, Sivasagar, Tinsukia
8	Kola Joha 2	Sivasagar*
9	Kola Kunkuni Joha	Sonitpur*, Lakhimpur, Jorhat, Tinsukia
10	Maniki Madhuri	Sonitpur*, Jorhat, Sivasagar
11	Podumoni Joha	Sonitpur*, Lakhimpur
12	Ranga Bhaboli Joha	Jorhat*
13	Rani Kajol	Sonitpur*
14	Ronga Joha	Sivasagar*, Jorhat
15	Tulsi Joha	Jorhat*

*District from where the accession was collected

relationship among morphological traits which contributes to aroma quality.

In the present study, aromatic germplasms were collected from different pockets of Upper Brahmaputra valley of Assam and characterized the morphological traits and found out their correlation with aroma in order to develop a strategy for improvement of Joha rice.

Materials and Methods

Chemicals

Standard 2-Acetyl-1-pyrroline (Make: Clearsynth:CS-O-00920) in 10% Toluene was purchased from Clearsynth Labs. Ltd., Mumbai, India. All solvents used for extraction process were Merck's high-purity SupraSolv® solvents.

Exploration and collection of Plant Material

A survey was conducted during 2016-2018 in different rice cultivating regions of Upper Brahmaputra Valley, Assam for native aromatic landraces of rice cultivated by local farmers. During this period, 15 landraces of Joha rice varieties were collected, which were then cultivated under uniform field conditions to observe the morphological parameters. The grains were harvested after ripening (Table-1 and Fig. 1).

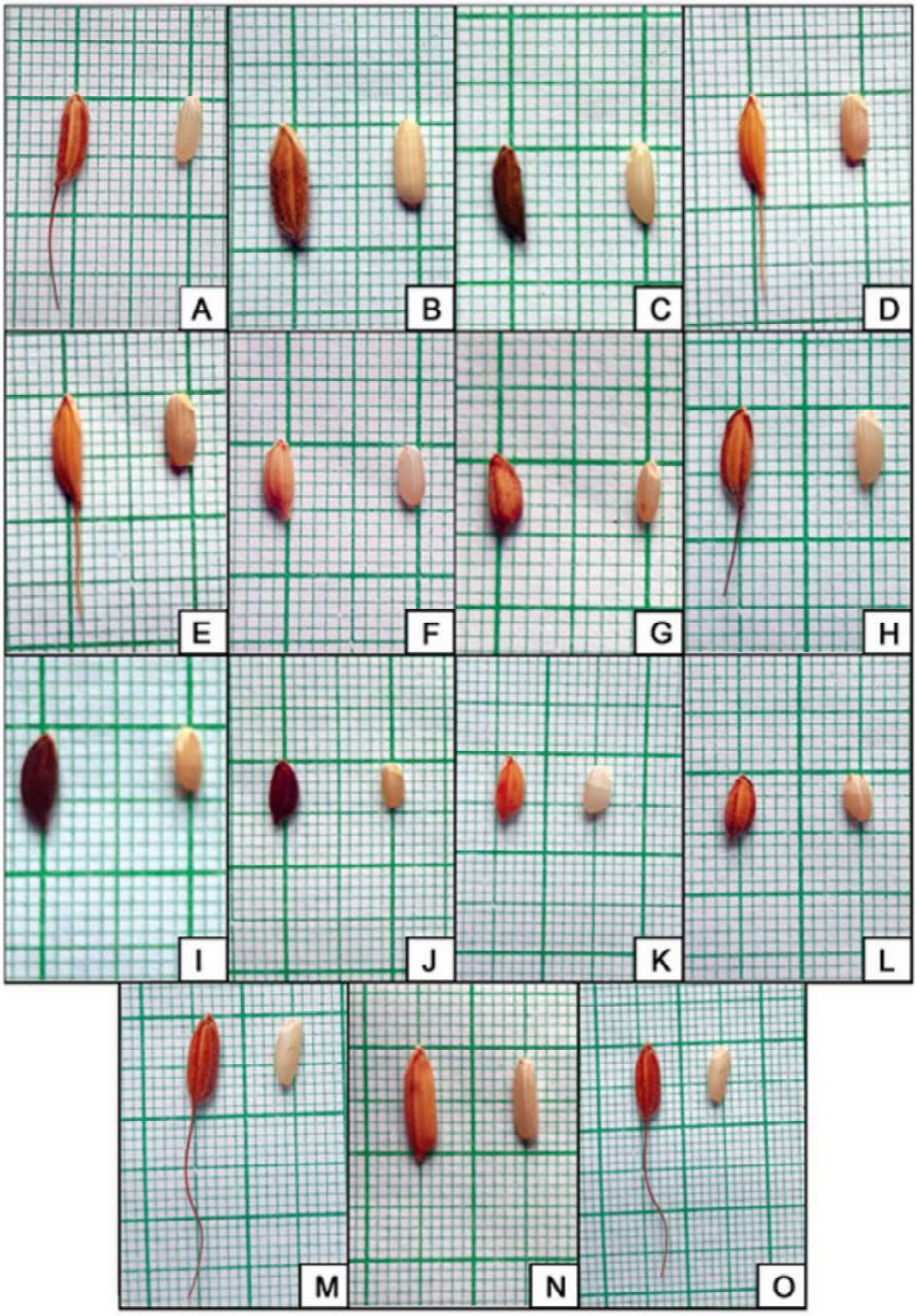

Fig.1 : Corticoted and decorticated grain of (A) Ronga Joha, (B) Joha Bora, (C) Kola Joha 1, (D) Kola Joha 2, (E) Boga Bhaboli Joha , (F) Ronga Bhaboli Joha, (G) Manikimadhuri Joha, (H) Boga Kunkuni Joha, (I) Boga Kon Joha, (J) Kola Kunkuni Joha, (K) Ranikajol Joha, (L) Dangor Joha, (M) Tulsi Joha, (N) Joha Bora and (O) Podumoni Joha.

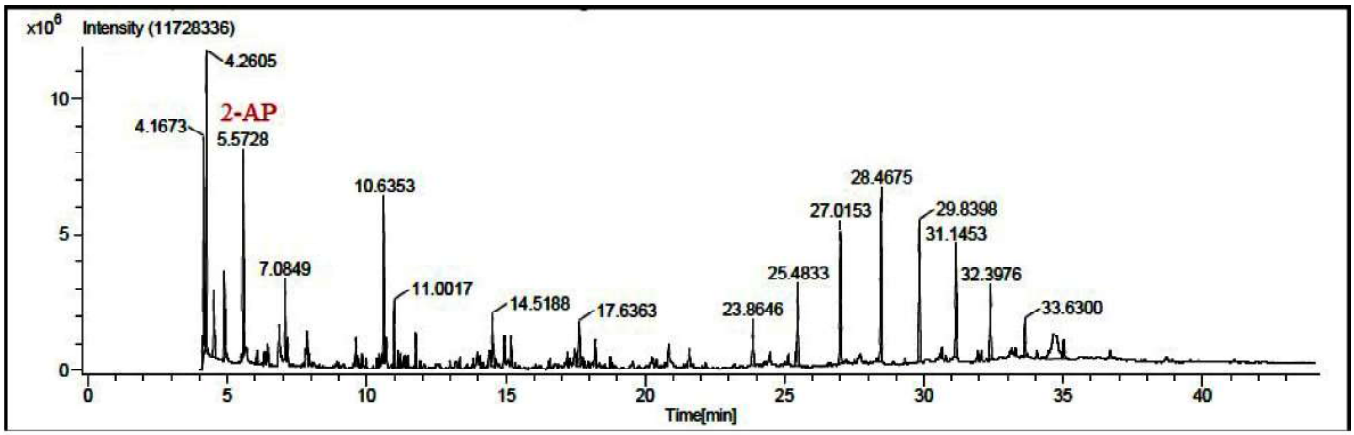

Fig. 2: GC chromatogram for volatile compounds in the grain of Dangor Joha.

Study of morphological characters

54 DUS characters were taken into account to study the morphological diverseness among the collected landraces and were computed as numerical notes as per DUS guidelines, Directorate of Rice Research, India[18]. The landraces were assessed for leaf, stem, panicles and grain characters during the period 2018-2021 for three consecutive years and was recorded.

Solvent Extraction of 2AP from grains of Joha

Joha rice grains were crushed and grounded to rice flour. About 50 g of these freshly prepared rice flour was packed in a thimble filter and bathed in 200 mL of analytical grade methanol for 24 h, followed by steam distillation in a soxhlet extractor continuously for 48 hrs. The extract was concentrated to10 mL in a rotary evaporator under reduced pressure and temperature around 40^0C. The solvent extract was then filtered through a sterile syringe filter (PTFE membrane) of pore size 0.45 µm and the same was concentrated to 1 mL by air drying.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-DIP (direct insertion probe was used to insert the sample into the GC MS system (JMS-T100GCV HPGC – ToF MS, Make-Jeol). The column used was a capillary column (DB-5ms, Make-Agilent Technologies), standard nonpolar with column length of 30 m and column internal diameter of 0.25 mm and film thickness of 0.2 µm. Helium was used as the internal carrier gas. The operation of mass spectrometer was carried in electron impact (EI) mode, with electron energy of 70 eV. The volatile compounds were then screened by ionizing volt of 70[V] and detector volt of 2250[V] and TOF for 45 minutes with the mass range (m/z) of 10-350.

Quantification of 2-Acetyl-1-pyrroline GC-MS was used to detect for the presence of the 2-Acetyl-1-pyrroline in term of existence percentage in chromatogram. EI mass spectrum of 2-Acetyl-1-pyrroline has molecular weight of 111 with NIST number of 281508. It shows total of 19 m/z peaks in GC-MS chromatogram of 2AP of which the 10 largest peaks for m/z are 43, 41, 42, 83, 39, 44, 69, 68, 111 and 37 (NIST Mass Spectrometry Data Center). A standard of 10% 2-Acetyl-1-pyrroline was used to compare for the existence percentage with the area of the peak for references to approximate standard concentration.

Correlation of 2-Acetyl-1-pyrroline with morphological characters

The amount of 2-AP estimated in 1 kg of grain was computed along with morphological character notes in PAST (PAleontological STatistics) version 4.03 program. 2AP content was correlated with the morphological character notes using linear correlation statistics (Pearson coefficient) to understand the relationship between 2AP accumulation in the grain and morphological characters[2,19].

Results and Discussion

Quantification of 2-acetyl-1-pyrroline

Quantification of 2AP among the Joha landraces using a standard 2AP of 10 µg/ml in Toluene as a reference, showed the distribution of 2AP across all the landraces. The highest peak for 2AP was observed at retention time of about 5.57 min (Fig. 2) with EI MS showing 19 m/z peaks of which the 10 largest peaks for m/z are 43, 41, 42, 83, 39, 44, 69, 68, 111 and 37 which corresponds to molecular weight of 111 with NIST number of 281508 (Fig. 3).

Average concentration of 2-Acetyl-1-pyrroline among all the landraces of Joha was found to be approximately 38.94 ìg/kg. 2AP was found highest in Dangor Joha followed by Kola Kunkuni Joha and Kola

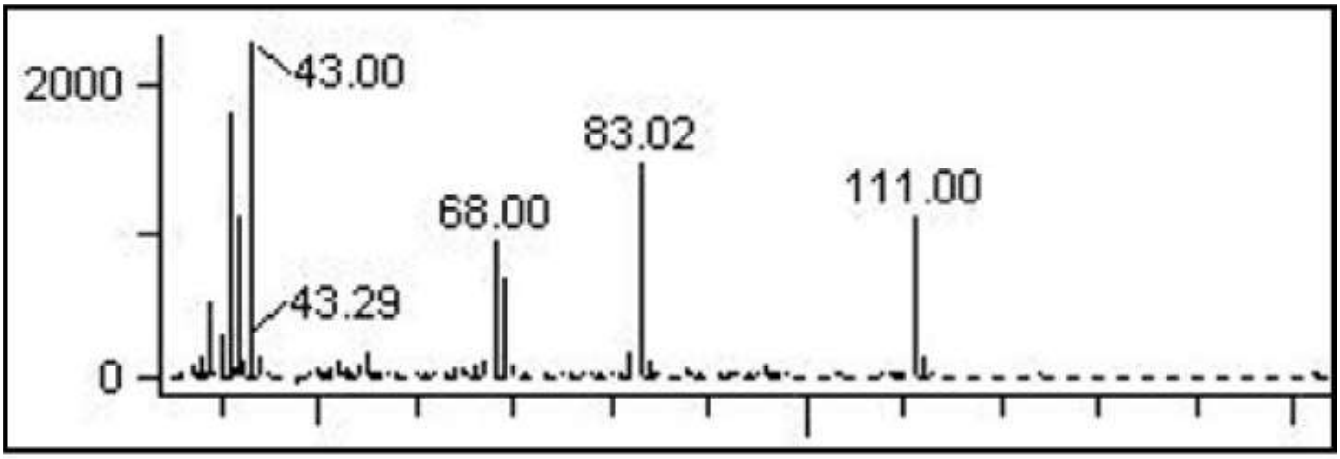

Fig. 3: MS of 2-Acetyl-1-pyrroline at time 5.57 min and molecular weight 111.

Joha 1 as 78.667 µg/kg, 64.615 µg/kg and 58.28 µg/kg respectively. It was least observed in Tulsi Joha, Boga Kon Joha and Ranikajol Joha with 2AP concentration of 19.76 µg/kg, 16.915 µg/kg and 14.465 µg/kg respectively (Fig. 4). This result shows strong evidence of the presence of 2AP in Joha rice, and it plays a crucial role in imparting aroma character in Joha rice. Dangor Joha, Kola Kunkuni Joha and Kola Joha 1 had higher accumulation of 2AP in their grain and thus had a stronger aroma in comparison with the other joha landraces. A similar result was also reported[2], in other aromatic and non-aromatic rice cultivars for analysing the volatile compound, where 2AP was absent in the later. Workers[20] quantified 2AP in boiled aromatic rice and reported about 146 µg/kg, 113 µg/kg and 80 µg/kg of 2AP in three fragrant rice namely basmati, Thai jasmine and Sintanur.

Correlation of 2AP content with morphological characters

When studying the correlation of morphological traits among the landraces, of the 52 morphological characters only 30 of these showed polymorphisms. Correlation analysis of the availability of 2AP with all the polymorphic morphological characters show that the 2AP concentration shows a strong positive correlation (Pearson coefficient r=) of 0.66501, 0.57017 and 0.50 with pubescence of leaf blade surface, width of leaf blade and density of pubescence of the lemma respectively (Fig. 5). Lengths of stem have a positive correlation with 2AP accumulation in rice grain. The correlation relationship between 2AP concentration and other morphological character decreases as medium Pearson's correlation coefficient of 0.4 for ligule colour, lemma & palae colour, spikelet colour, colour of leaf and sterile lemma colour.

Similarly, the concentration of 2AP with grain length shows a medium negative correlation of -0.3396 (Pearson coefficient). Morphological character such as basal leaf sheath colour, anthocyanin colouration of leaf sheath, grain weight, grain width, maturation time, attitude of panicle branches and stem length have very small positive correlation (below +0.29) with 2AP accumulation.

Earlier, correlation analysis was studied between agro-morphology and grain quality of aromatic rice[6,7,11,14,15], but the present study focuses on the correlation of 2AP content with agro-morphology for the first time in Joha rice. The present findings show a positive correlation of stem length, days to maturity of panicles, width of blade, pubescence on blade and lemma with 2AP in grain. Workers[4] also reported positive correlation of aroma level in aromatic rice of Odisha, with plant height and days to maturity of panicles which is at par with the present finding.

Increase in 2AP content in joha showed a negative correlation with panicle numbers per plant, panicle distribution and secondary branching of panicles which directly reflects to low grain yield. This may be due to the non-functional BADH2 gene responsible for 2AP accumulation in aromatic rice which results in under development of pollen tube[1,9]. Some have reported negative correlation between aroma and grains per panicles as well as yield[4]. High aroma content in aromatic rice is determined by physiological process leading to low grain yield. Findings of the present study affirm the importance of morphological traits in selection of landraces with higher 2-Acetyl-1-pyrroline content in Joha rice.

Conclusion

The present investigation provides the information of accumulation of 2-Acetyl-1-pyrroline in the grain of joha rice landraces with special reference to correlation of the aroma nature with agro-morphological and qualitative traits. With the introduction of high yielding

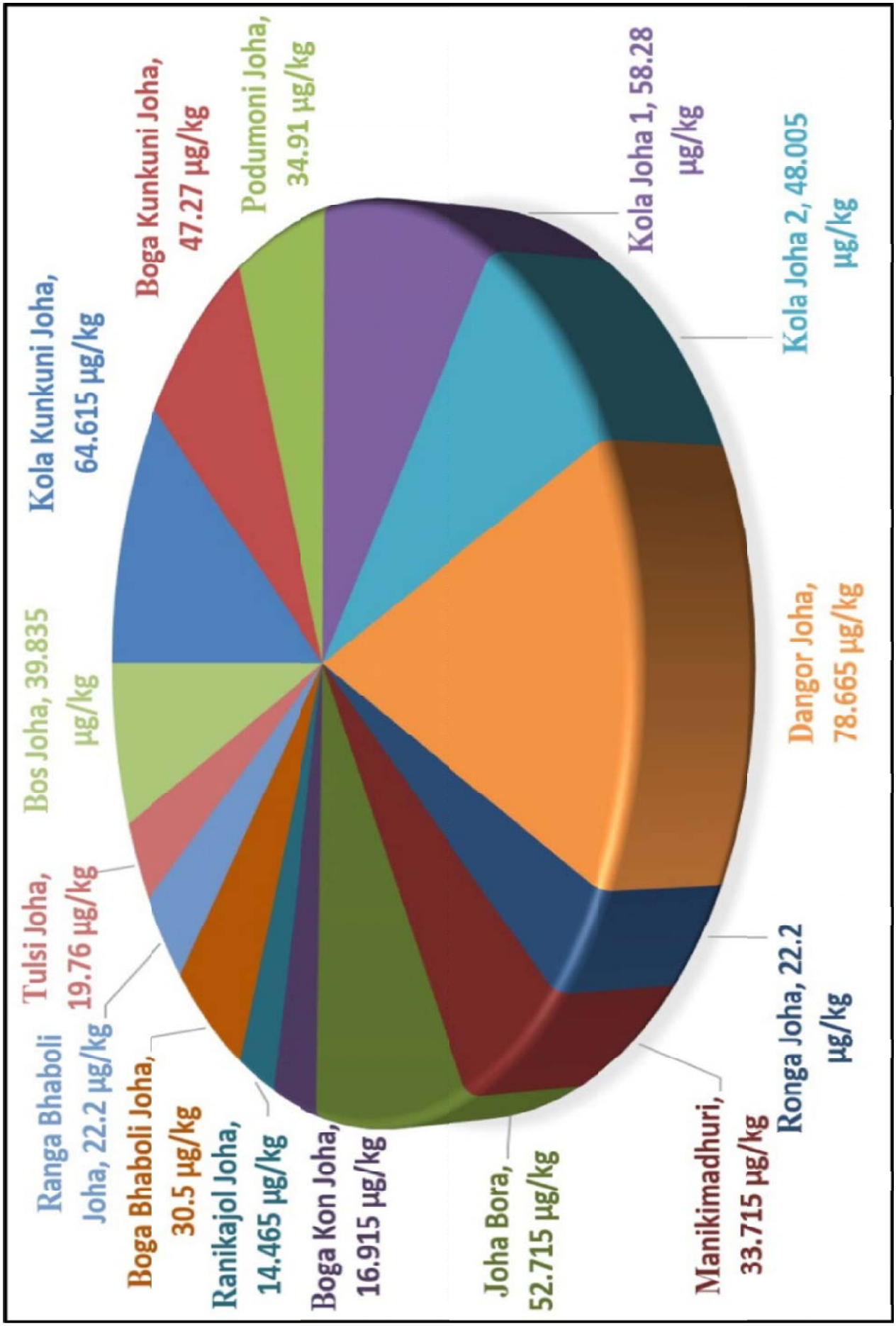

Fig. 4: Distribution of 2AP in 1 kg of rice grain, across the 15 landraces of Joha rice using GCMS analysis

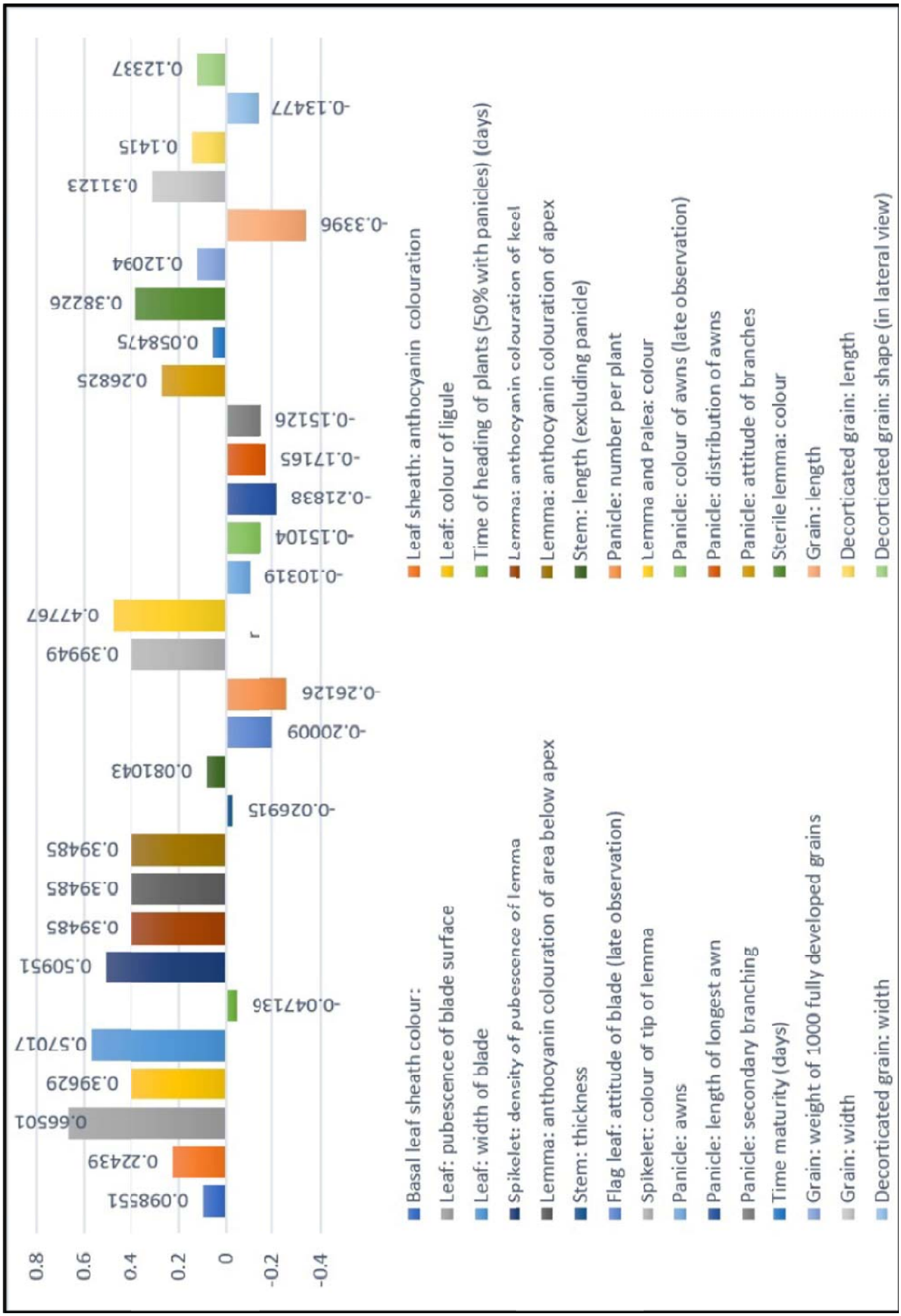

Fig 5: Pearson's Coefficient Correlation (r) of 2-Acetyl-1-Pyrroline content in grain with the polymorphic morphological characters

varieties of rice, it has been challenging for the local farmers to cope up with the economy to cultivate these low yield endemic aromatic rice landraces. Thus, the conservation of these landraces is a need of the hour in the present scenario of global argo-economy.

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