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ORIGINAL ARTICLE **OPEN ACCESS**

Estimation of amino acids in pigmented rice landraces from Kumaun region of Uttarakhand

Ajay Kumar[1], Netrapal Sharma[1], Kulbhushan Kumar[1], Ankit Kumar[2], Shikha Bora[1] and Satpal Singh Bisht[1]
[1]Department of Zoology, D. S. B. Campus Kumaun University Nainital
Uttarakhand, Pin no-263002
[2] Department of Pharmaceutical Sciences, Bhimtal Campus, Kumaun University, Nainital, Uttarakhand, India
*Corresponding Author's Email ID: np_biotech53@rediffmail.com
ORCID ID: 0000-0003-3997-0453

ABSTRACT
Seeds accumulate proteins in protein storage vacuoles (PSVs) and these seed proteins undergo enzymatic denaturation by lipoxygenase and non-enzymatically by the oxidation of polyunsaturated fatty acids. Long-term storage can cause denaturation of seed storage proteins. Amino acids of cereal grains control the metabolism, diseases and play a crucial role in seed germination. The present study highlights the amino acid composition and germination efficiency in the stored seeds of pigmented rice landraces from the Kumaun region of India. All seed samples yielded 1.64µg/µl to 2.19µg/µl of total buffer soluble protein. Amino acid analyses performed by Whatman chromatography paper and the maximum number of amino acids were present in the ASP and APP1, i.e., four amino acids in each. Germination efficiency of ten colored rice landraces was also studied. When stored at a low temperature, no significant effect was observed.
Keywords: Rice landraces, Seed storage proteins, Paper chromatography, Amino acids.

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INTRODUCTION

Rice is known as a staple food throughout the world. It provides food, financial and social security to the farmers in developing countries like India. Approximately 1, 20,000 varieties of rice exist globally, out of which 40,000 cultivars are grown [1]. Global rice production projected to reach 46.3 million tons in 2020 [2]. The productivity of rice in the mountains is very low compared to the plains of Uttarakhand. More than 103 landraces of rice found in Uttarakhand [3] but very few studies have been made as of the date. Most of the villagers of the Kumaon region cultivate rice traditionally and use local agricultural methods for cultivation. Grown-up landraces are organic rice usually grown by small farmers. The annual rice production of Uttarakhand is around 5.94 lakh tonnes and productivity is 21.20 quintals per hectare [4]. The Western Himalayan region of India has a rich source of rice genetic resources. Differences in rice varieties are classified in many aspects, based on their morphology, nutrient composition, aromatic properties, texture and physical parameters.

Rice has many commercial facets including the cosmetic and wine industry [5]. Pigmented rice varieties at higher elevations are of great nutraceutical value and contain cold-tolerant genes [1]. Their export capacity is increasing due to demand in the world market [3].

Rice seed contains carbohydrates more abundantly apart from proteins, minerals, fibers and vitamins. The nutritional quality and germination potential of seeds are related to temperature, oxygen, seed content, dryness and air humidity [6]. Moisture increases oxidization and helps in the generation of free radicals and damages the lipids and nucleic acids. DNA damage leads to disturbed transcription and translation processes. Finally, the oxidization of amino acids occurs [7, 8, 9]. Lipids, starch and proteins are stored in the endosperm of rice seeds and bounded by the aleurone layer [10] and genetic information is stored inside the embryo and organizes germination. Free radical-mediated lipid peroxidation, inactivation, or protein degradation is related to seed feasibility and vigour. Proteases convert the seed storage proteins into smaller peptides by hydrolysis [10].

During the present investigation, high altitude rice landraces were studied for protein and amino acids present in these less known varieties by a double beam UV-Vis spectrophotometer, paper chromatography; their seeds germination potential was also investigated.

MATERIAL AND METHODS

Rice cultivar collected from local farmers of Pithoragarh, Almora and Bageshwar district of Kumaun region (Figure 1 and 2). The collected samples were coded based on their collection sites. The chemicals and reagents purchased from SRL Chemicals include n-butanol, Glacial acetic acid, Coomassie Brilliant Blue G-250, Bovine serum albumin (BSA), sodium citrate, HCl, benzoic acid and standard amino acid kit for reference.

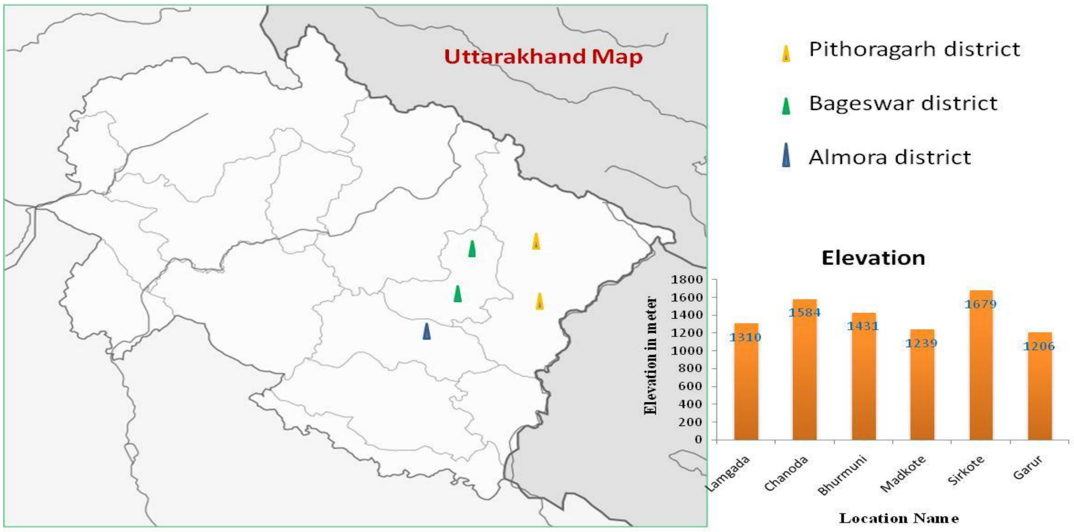

Figure 1. Location of collection sites with their altitude (m.asl)

Figure 2. Pigmented rice landraces collected from Kumaun region, India

Moisture content

The moisture content of samples was determined using the oven drying method. 6g of seed powder was weighed and dried in an oven at a constant temperature of 100^0 C for 6 hours. The final weight was measured and the moisture content in percentage was calculated Raghuramulu *et al.* [11].

Moisture content (%)= (W2- W3/W2- W1) X 100

Where,

W1 = weight of empty crucible

W2 = weight of sample before drying

W3 = weight of sample after drying

Extraction of Total amino acid
10 mg of defatted rice seed powder was hydrolyzed using 1ml of 6 N HCL and kept in an oven. For 24 hrs at 110˚ C so those amino acids could breakdown the intact protein ultimately into amino acid residues. HCL was evaporated at 65° C using a burner and placed into a close chamber for 6 hours. Finally, the dried sample was dissolved in 1ml Na-S™ buffer (2% sodium citrate, 1% HCl, 0.1% benzoic acid)[12].
Free amino acid
25 mg rice powder was utterly mixed in 500L Na-STM buffer and ultrasonically extracted for around 15 minutes. The supernatant was collected and filtered using a 0.45-m nylon membrane syringe filter after centrifugation for 10 minutes at 16,000 rpm [12].
Spectrophotometric analysis of total seed storage protein
0.1g defatted seed powder was mixed with 1ml of buffer (0.625 M Tris-cl, 2% SDS, 5% beta-mercaptoethanol, 10% glycerol at pH 6.8) and the mixture was incubated for 10 minutes in a water bath at 100 degrees Celsius and centrifuged at 10000 rpm for 15 minutes in 4ºC (Sharma *et al.*[13], Kumar *et al.* [14]). The supernatant was collected to check the protein concentration by Bradford assay [15] [16].
Paper Chromatography analysis of total and free amino acid
Chromatographic analysis was performed according to Jain *et al.* [17] with minor modification. Solvent system was prepared using n-butanol, acetic acid and water in a ratio of (12:3:5), 1.5% w/v ninhydrin in n-butanol and was mixed with 0.3% v/v acetic acid. After that, the solvent was poured into an airtight glass chamber and chromatographic paper; the solvent was allowed to run 3/4 on paper followed by air drying at room temperature and after drying ninhydrin was sprayed on the paper, followed by air-drying and spots were observed after 5 minutes of incubation at 100°C [17].

RESULTS AND DISCUSSION
Commercially important varieties of crops are cultivated in the plains and Shiwalik region of the state. However, many rice landraces are cultivated in the higher Himalayan regions. The coloration in the rice varieties is due to anthocyanins, β-carotene and phenolic compounds. The allocation of these compounds is affected by geographical location, soil type, other climatic conditions and genetic makeup of the cultivars [18].These landraces are dependent on monsoon due to the lack of a proper irrigation system; Morphometric characters of ten pigmented rice landraces a summarized in Table 1.

Table 1. Morphometric characterization of the grains of selected ten pigmented rice landraces

Rice Landraces	Name of collection sites	Kernel colour (by Munsell soil color charts)	Seed coat colour (by Munsell soil color charts)	Elevation (in Meters.)
AGB	Bhurmuni (Pithoragarh)	5YR,6/6	10YR,8/6	1431
ALA	Garur (Bageshwar)	2.5YR,5/8	10YR,8/6	1206
ATA	Bhurmuni(Pithoragarh)	5YR, 6/8	10YR,8/6	1431
AMP	Madkote (Pithoragarh)	2.5YR,5/8	10YR,8/4	1239
ABP	Chanoda(Bageshwar)	7.5YR,6/8	10YR,8/3	1584
ASP	Sirkote (Bageshwar)	10YR,7/6	10YR,8/6	1679
APP1	Lamgada(Almora)	5YR,6/6	10YR,7/8	1310
AVA	Lamgada(Almora)	2.5YR,5/8	10YR,8/6	1310
ALP	Manitundi(Pithoragarh)	5Y,6/6	10YR,4/6	1431
APP2	Garur(Bageshwar)	7.5YR,6/8	10YR,8/3	1206

The protein estimation was performed as per Bradford method (Bradford [15]); and the highest protein content was yielded by the sample APP2 collected from Garur (Bageshwar) (Figure 3). The total amino acids present in ten pigmented landraces of *Oryza sativa* are presented in Table 3**.**

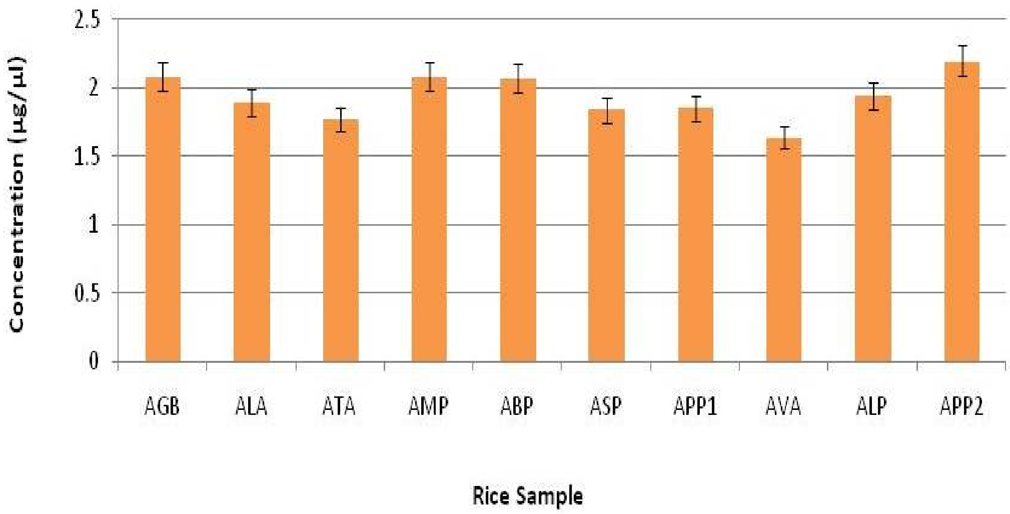

Figure 3. Total buffer soluble protein in ten rice cultivars

Table 2. Rf value of standard amino acids (Rao *et al.,* [19], http://www.reachdevices.com/TLC_aminoacids.html).

Standard Amino Acids	Rf value	Rf Value (Range)
Alanine	0.32	0.30 (0.46)
Arginine	0.11	0.16 (0.32
Asparagine	-	0.21 (0.24)
Aspartic acid	0.14	0.24 (0.29)
Cystine	0.03	0.14 (0.15)
Glutamic acid	0.25	0.31 (0.35)
Glycine	0.19	0.25 (0.26
Histidine, HCl*	0.11	0.12 (0.32)
Isoleucine	0.65	0.53 (0.73)
Leucine	0-85	0.61 (0.71)
Lysine, HCl*	0-11	0.12 (0.24)
Methionine	0-82	0.51 (0.60)
Ornithine, HBr*	0.3043	0.3043
Proline	0.40	0.24 (0.48)
Serine	0.19	0.16 (0.25)
Threonine	0-35	0.30 (0.36)
Tryptophan	-	0.61 (0.57)
Tyrosine	0-53	0.55 (0.44)
Valine	0.62	0.44 (0.66)

Amino acids in paper chromatography of rice samples detected are presented in the chromatograms (Figure 4, Table 3 total amino acids and Figure 5 Table 4 free amino acids). Usually, amino acids are colorless. The spots are shown on the plate after the reaction of ninhydrin with amino acid and measurement of a spot on activated Whatman no1 chromatography paper. The reproducible values $\mathbf{R_F}$ (Rf means relative to front) calculated as described by C. K. Kokate [20].

RF value= Total Distance traveled by solute/Total distance traveled by the solvent

Table 3. Total amino acids present in ten landraces of Oryza *sativa*

Sample	R_F value for total amino acids	Amino acid
AGB	0.1304 0.4347 0.5565	L-Lysine Alanine Tyrosine
ALA	0.1478	L-Lysine
ATA	0.1304 0.4347 0.5652	L-Lysine Alanine Tyrosine
AMP	0.1304 0.4347 0.5652	L-Lysine Alanine Tyrosine
ABP	0.1565 0.5652	L-Lysine Tyrosine
ASP	0.1304 0.4608 0.5304 0.6782	L-Lysine Alanine Tyrosine Leucine
APP1	0.1304 0.4521 0.5913 0.6956	L-Lysine Alanine L-Proline Leucine
AVA	0.1304 0.4347 0.5565 0.6782	L-Lysine Alanine Tyrosine Leucine
ALP	0.1304	L-Lysine
APP2	0.1304	L-Lysine

The maximum number of total amino acid spotted in ASP, APP1 and AVA four amino acids and lowest number of amino acids were found in ALA, ALP and APP2 (one amino acid).

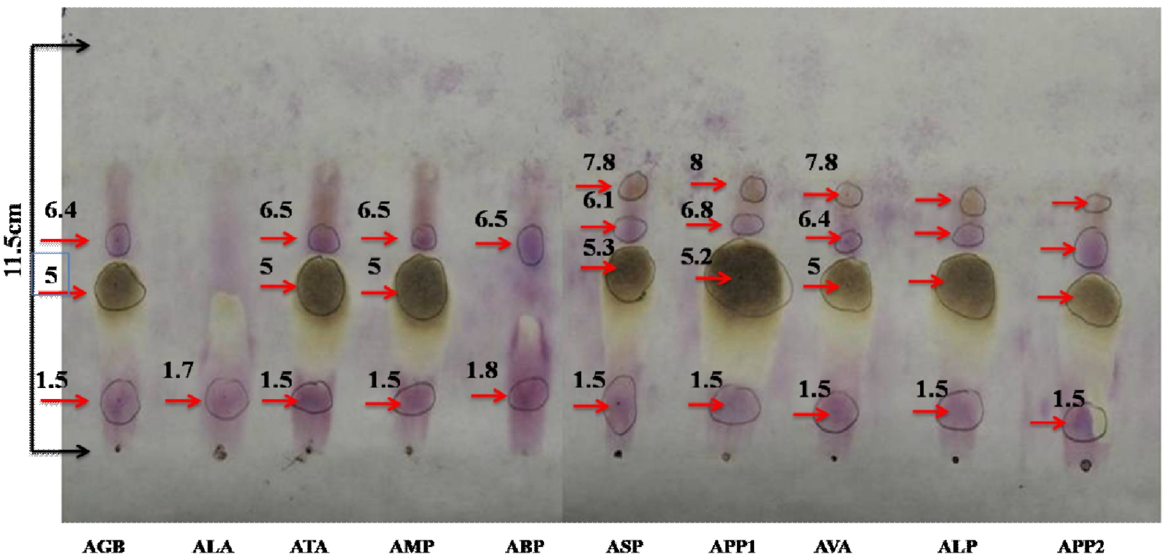

Figure 4. Paper Chromatogram of ten rice landraces (Total amino acid)

Free amino acid

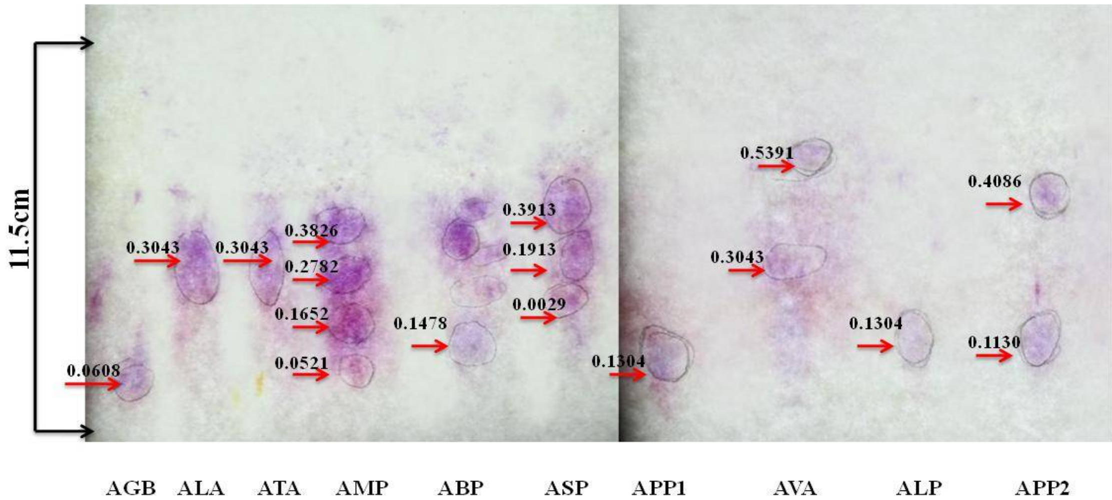

Figure 5. Paper Chromatogram of ten landraces (Free amino acid)

Many amino acids were found in ten pigmented local landraces during the present study, i.e., Cystine, Ornithine, Histidine, Aspartic acid, Tryptophan, Amino-n-butyric acid Alanine, Tyrosine, Glutamic Acid, Serine, Leucine and Valine. At the same time, other studies were relatively different as they reported a comparatively a smaller number of amino acids from rice landraces [21] (Figure 5).

Table 4. Identification of free amino acids using Paper Chromatography

Rice Landraces	R_F value for free amino acids	Amino acid
AGB	0.0608	L-Cystine
ALA	0.3043	L-Ornithine
ATA	0.3043	L-Ornithine
AMP	0.0521 0.1652 0.3826 0.2782	Histidine Aspartic acid Tryptophan Amino-n-butyric acid
ABP	0.1478	Lysine
ASP	0.1913 0.0029 0.3913	Alanine Tyrosine Tryptophan
APP1	0.1304	Glutamic Acid
AVA	0.3043 0.5391	Ornithine Serine
ALP	0.1304	Glutamic Acid
APP2	0.1130 0.4086	Leucine Valine

Sample number 5, *Oryza sativa* (ABP) shows the highest concentration of total amino acid and minimum amount in sample number 1, *Oryza Sativa* (AGB) was observed; other samples show the average amount of amino acids. The highest concentration of free amino acid in *Oryza sativa* (ALP) and the rest of the samples show almost equal free amino acid (Figure 6).

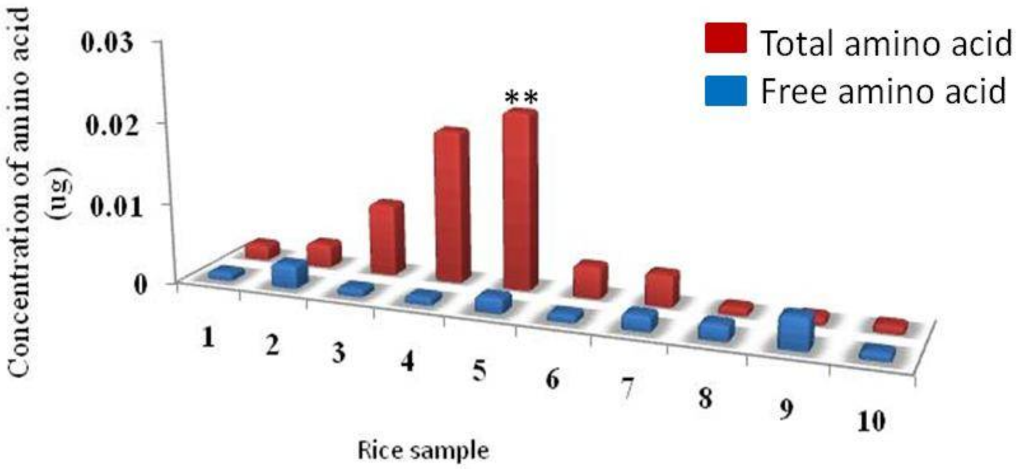

Figure 6. Concentration of total and free amino acid of rice cultivars

The germination performance of all ten landraces was analyzed and it was observed that the germination percentage is excellent even in the laboratory condition the landrace APP1 the germination success was 96% the landraces ATA showed a minimum of 45%. All seeds germinated successfully even without any physical and chemical treatment (Figure 7).

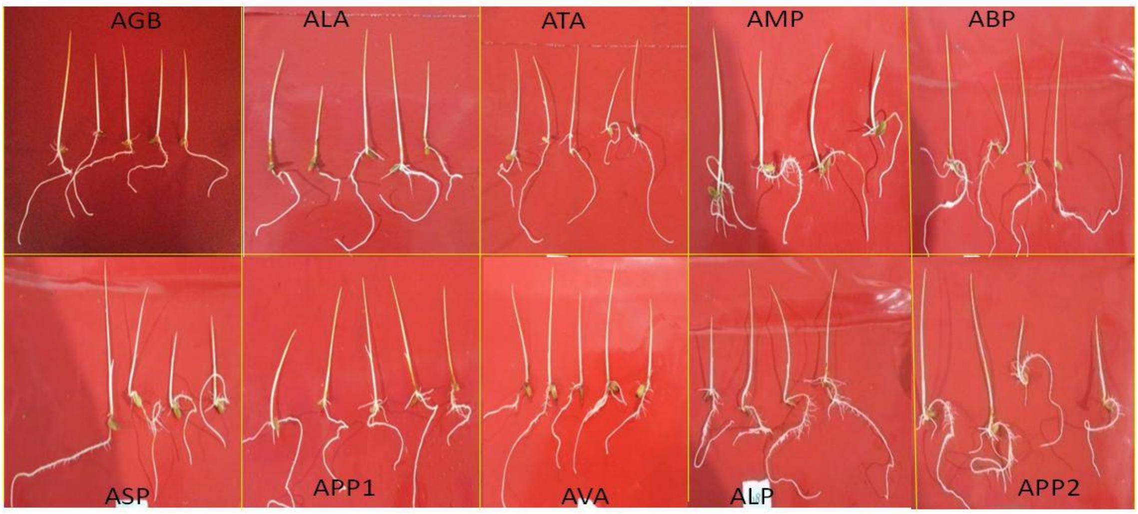

Figure 7. Germination performance of ten local rice landraces

The sum of the seed's qualities that impact the degree of germination and seedling emergence activity and performance of the seed or seed batch is known as seed vigour. The ISTA Congress established the definition of seed vigour in 1977. Seeds that are low in vigour, on the other hand, will produce weaker seedlings that are more vulnerable to assault, contamination, and other environmental concerns. Seed lots with a higher seed vigour index are thought to be more vigorous [22].The Association of Official Seed Analysts (1990) instruction followed on the recording of rice seeds germination. Germination percentage calculated by Ratio of the germinated seed of the whole number seed. Seed vigour index is calculated by multiplying germination (%) and seedling length. Seed lots with a higher seed vigour index are thought to be more vigorous [22]. In the laboratory, changes in physiological characteristics of seed batches can be demonstrated. It was suggested that the phrase characterize seed performance when planted in the field [23].The vigour index of ten pigmented landraces is presented in Table 5.

Table 5. Germination, Moisture and Vigor percentage of an examined sample of Rice landraces (http://www.knowledgebank.irri.org/step-by-step-production/pre-planting/seed quality?tmpl=component-category & print=1).

Sample	Germination %	Ratio	Vigour Index	Moisture %
AGB	82	68.05	3.526	18.05 ± 0.047081
ALA	52	33.3	2.5584	13.85 ± 0.210594
ATA	45	28.66	1.773	12.895 ± 0.31808
AMP	56	42.05	3.0072	9.335 ± 0.115217
ABP	89	65.45	4.6458	8.9625 ± 0.147895
ASP	73	54.5	1.5184	7.1825 ± 0.113385
APP1	96	73.75	4.8	8.1625 ± 0.227317
AVA	92	89.8	4.2872	20.43 ±0.257132
ALP	96	57.85	4.968	7.3725 ±0.554908
APP2	69	45.3	3.6225	13.1925 ±0.203034

While analyzing the various amino acids present in various pigmented landraces, fourteen amino acids present in one or another viz amino acids (AA) are organic substances containing both amino and acid groups. Apart from glycine, all AA have an asymmetric carbon and exhibit optical activity. The absolute configuration of AA (L- or D-isomers) is defined concerning glyceraldehydes. Except for Proline, all protein AA have a primary amino and a carboxyl linked to the a-carbon atom (hence a-AA). In b-AA (e.g., taurine and b-alanine), an amino group links to the b-carbon atom and post-translationally modified AA occurs in some proteins [24]. Amino acids are cell signaling molecules as well as the regulators of many other organic phenomenons.

The amino acids present in the pigmented rice varieties of Kumaun region are summarized with their sources and therapeutic value, presence of these amino acids makes these landraces a good nutraceutical source.

Tryptophan: Tryptophan is a vital amino acid usually present in food with high protein i.e., peanuts, pumpkin and sesame seeds, turkey, whole chicken milk, tofu and soy etc. It is an in vivo precursor for nicotinamide (vitamin B6), serotonin, melatonin, tryptamine, kynurenine, 3-hydroxykynurenine, quinolinic and xanthurenic acids, among other beneficial chemicals [25]. The amount of free Tryptophan in transgenic rice seeds was roughly twice that of wild-type rice seeds [26]. As a result of this finding, the authors hypothesized that the tryptophan content of rice seeds might be enhanced trans genetically to improve the nutritional value of human and animal meals. During the present study Tryptophan was reported in pigmented rice landraces *Oryza sativa* ASP. Similarly other amino acids which those have high medicinal and therapeutic value reported from ten pigmented rice landraces amino acids Histidine, Aspartic acid, Tryptophan, Amino-n-butyric acid found in AMP and total amino acid present in ASP L-Lysine, Alanine, Tyrosine, Leucine, APP1 L-Lysine, Alanine, L-Proline, Leucine another record with three, two and one each in ALP, APP2 (Table 3).

Alanine: Alanine is reported from six out of ten pigmented rice landraces. Tryptophan and vitamin B-6 are broken down using alanine to inhibit the activity of gluconeogenesis. It is a source of energy for the central nervous system and muscles. It helps the body to use glucose and boosts the immune system. A daily intake of 2-5 grams is advised. Although too much beta-alanine can produce tingling in the skin, it is regarded as a safe and helpful supplement for improving athletic performance. Some protein-rich plant foods have supplement alanine and excellent sources included meat and poultry, fish, eggs and dairy products [27].

Tyrosine: The present study tyrosine reported in three landraces, namely *Oryza sativa* (AGB, ATA and AMP). Tyrosine is an essential component for producing various neuro-chemicals called neurotransmitters including epinephrine, norepinephrine and dopamine. These neurotransmitters have the potential to assist people who are depressed. According to a review of considerable research, tyrosine may be helpful in treating mild-to-moderate depression [28].Tyrosine is found in dairy products, casein, meats, fish, eggs, nuts, beans, oats and wheat.

Lysine: Out of the ten pigmented landraces Lysine was present in all samples, indicating the richness of lysine in pigmented rice landraces. Lysine is essential for protein synthesis, hormone, enzyme production, and calcium absorption. It is also necessary for energy production, immunological function and bone matrix formation (collagen and elastin). The human body cannot make Lysine so it must be taken in the form of meat, fish, dairy, eggs and some soy plants.

Leucine: In the present study three pigmented rice varieties contain leucine, i.e., *Oryza sativa* (ASP, APP1 and AVA). Leucine and valine is an essential branched-chain amino acid for protein synthesis and muscle repair. It also aids blood sugar regulation, wound healing and the production of growth hormones. Leucine also assists the control of blood sugar levels. It is thought to do so through improving insulin responsiveness and promoting glucose uptake in the human body. Some amino acids such as leucine can improve insulin signaling and glycemic control [29, 30, 31, 32].

Proline: Proline was recorded from one variety of pigment rice out of ten studied during the present investigation. It is necessary for protein synthesis and metabolism and wound healing, antioxidative reactions, and immunological responses. Proline usually found in various fruits and vegetables. Some Proline-Rich Foods are Asparagus, mushrooms, cabbage, bell peppers, strawberries, broccoli, and citrus fruits.

Valine: Valine was present in one pigmented rice during the present study i.e., *Oryza sativa* (APP2). Valine belongs to the branched-chain amino acid. It has wide applications within the field of pharmaceutical and food industries. Valine is a nutrient that aids in muscle growth and regeneration as well as energy production. It is an important aminoalkanoic acid found in proteins important for optimal growth in infants, children and adults. The lack of L-valine may influence the expansion of the body cause neuropathic obstacles anemia, Obesity Type 2 Diabetes Mellitus, Inflammatory Bowel Disease, Atherosclerosis and colon cancer [33, 34, 35].

Glutamic Acid: During the present study, glutamic acid was reported from two varieties of pigmented rice Oryza sativa (APP1 and ALP). Glutamic acid functions like endogenic anticancer agent and plays an important role in the tumor cell. Glutamic acid acts as a nitrogen donor in the nucleotide and amino acid biosynthesis and helps in the uptake of essential amino acids to maintain the activation of target rapamycin kinase [36]. Sources of glutamic acid include high-protein foods such as meat, poultry, fish, eggs and dairy products.

Serine: In this study serine reported from one variety of pigmented rice, Oryza sativa (AVA), making it an effective food and maintaining sugar balance. Serine plays a central role in cellular proliferation. Serine concentrations are high in all body fluids during early fetal development (measured by standard amino acid analysis and thus comprising both D and L-serine) [37]. Serine plays an important role in diabetic Retinopathy and retinal homeostasis. The major food source of Serine are soybeans, nuts (especially peanuts, almonds, and walnuts), eggs, chickpeas, lentils, meat and fish (especially shellfishes).

Ornithine: During the present study three varieties were observed as having ornithine i.e., *Oryza sativa* (ALA, ATA, AVA). Ornithine may help to perform better in sports and athletic performance (such as speed, strength, and power). Ornithine taken orally may reduce fatigue. Taking Ornithine in combination with Arginine also seems to improve strength and power in male weightlifters [38]. Ornithine mainly found in Food sources like Walnuts, Hazelnuts, Pecans, Peanuts, Almonds, Cashews and Brazilian Nuts.

Cystine: Cystine was reported from one variety of pigmented land rice *Oryza sativa* AGB during the present investigation. The body can make Cysteine from the amino acids Methionine and Serine. The human body produces Cysteine from an essential sulfur-containing amino acid known as Methionine. This amino acid is abundant in natural products like ricotta, cottage cheese, yogurt, sausage meat, chicken, turkey, duck, luncheon meats, wheat germ, granola, and oat flakes.

Histidine: One variety of pigmented landrace present in this amino acid i.e., *Oryza sativa* (AMP). Histidine produces histamines neurotransmitter that is important for an immunological response, digestion, sexual function and sleep-wake cycles. It is necessary to maintain the myelin sheath a protective barrier of nerve cells. Histidine helps in the treatment of rheumatoid arthritis and anemia in chronic renal failure patients [39]. Major Food sources of Histidine included meat, fish, poultry, nuts, seeds, and whole grains contain large amounts of Histidine.

Aspartic acid: During the present study it was observed that *Oryza sativa* (AMP) contains aspartic acid. Aspartic acid is a natural amino acid. Previous research suggests that it works mainly by increasing follicle-stimulating hormone and luteinizing hormone levels, the latter it stimulates Leydig cells in the testes to produce more testosterone [40]. It can boost low testosterone levels. Food sources of aspartic acid i.e., oysters, luncheon meats, sausage meat, sprouting seeds, oat flakes, avocado, asparagus, young sugarcane, molasses from sugar beets, Dietary supplements and aspartic acid itself or salts (such as magnesium aspartate).

Amino-n-butyric acid: Amino-n-butyric acid: Amino-n-butyric acid was reported from one pigmented rice variety i.e., Oryza sativa (AMP). Amino-n-butyric acid is a non-proteinogenic amino acid that is widely present in microorganisms, plants and vertebrates. It is function as an effective agent in lowering blood glucose, attenuating insulin resistance, stimulating insulin release and preventing pancreatic damage [41].

CONCLUSION
The findings and observation of the present study conclude that the pigmented rice variety of Kumaun region is very diverse in terms of amino acids, having a great nutritive value and therapeutic importance. High protein content and more amino acids are present in most of the selected pigmented landraces compared to the commonly grown varieties, therefore, these varieties need in depth investigations and their conservation, both In-situ and Ex-situ should be promoted to protect the gene pool of mountain Rice landraces. Therefore, it needs to be conserved using in modern genomic tools and techniques. Farmer should be promoted and aware of pigmented landraces therapeutic and medicinal value, including the higher cost of these landraces. Pigmented rice can be grown at a larger scale for commercial value as it was observed that farmers are cultivated their pigmented landraces in a minimal area for a particular purpose.

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CONFLICTS OF INTEREST
The authors affirm no conflict of interest.

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ETHICS STATEMENT
NA

INFORMED CONSENT
NA

DATA AVAILABILITY STATEMENT
Data is contained within the article.

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