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Research Article

Characterization of Nepalese rice (*Oryza sativa* L.) landraces for qualitative traits

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ABSTRACT

The characterization of rice (*Oryza sativa* L.) landraces enables to identify phenotypically unique variables which certainly aid in rice breeding program. So, an experiment was conducted in alpha designed to characterize 188 rice landraces from NAGRC (National Agriculture Genetic Resources center) Nepal for their qualitative agromorphologies in research farm of Agriculture and Forestry University (AFU), Rampur, Chitwan in 2020 AD. Twenty-nine qualitative variables viz; twelve leaf characters, six culm characters, four panicle character and seven grain characters were observed and 26 characters revealed diverse trait expressions for each variable in experimented 188 rice accessions. Two leaf characters namely ligule colour and flag leaf attitude for early observation and one grain character (stigma colour for early observation) showed no variation among studied rice accessions. The intensity of green colour of leaf blade, culm lodging resistance and culm habit, secondary branching of panicle, and lemma and palea colour, lemma apiculus colour and sterile lemma colour, elucidated the higher variation in studied characters. The distinction revealed in qualitative characters approves the presence of abundant phenotypic diversity in the landraces assemblage and that eventually signifies the efficient and effective utilization of landrace in rice breeding programs.

Keywords: Rice landraces; qualitative characters; rice breeding

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INTRODUCTION

Nepal is gifted with an ample diversity of rice germplasm with thousands of rice landraces showing variation. Nepal is a center of origin for rice. Rice samples of 500 years ago were found at Simraungardh, Bara (Mallick, 1981/82) which supports the statement that Nepal is one of the centers of rice diversity (Joshi, 2005). A total of 8389 rice accession are conserved by variety of domestic and global gene banks from Nepal and approximately, 2500 accession are categorized to different accession number by NAGRC/NARC, but remaining germplasm are not charaterized yet all (Kandel, 2018). Rice breeder of Nepal released only 86 rice varieties including 2 hybrids (F_1) in last 5 decades (SQCC, 2077) using mostly exotic advance lines. In addition to this, 71 rice varieties were registered since 2010 (SQCC, 2077). This illustrates quite low variety replacement rate (VRR) in Nepal. Although Nepal is a

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center of origin of rice comprising more than 25 hundred indigenous landraces, released rice varieties are extremely dependent on exotic germplasm >95% (Kandel, 2018) principally from IRRI. These considerable numbers of landraces can provide vital information for the breeding of crop and in the handling of genetic resources.

Indigenous rice landraces have been gradually fading out due to absolute use of semi-dwarf exotic advanced and homogeneous rice (Biswas *et al*., 2021). While farmers acquired high and uniform yielding, input responsive rice seeds from the market, they stopped saving their indigenous seed stocks. Over the years, we began losing the thousands of landraces that were portion of our food and agricultural heritage (Chaudhary *et al*., 2003). Amidst current situation of global pandemic, grain paucity, alarming increasing population and snowballing climate change; it is coercing to intensify the grain (rice) production. This compellable situation can only be resolved by developing better rice cultivars with utilization of our local germplasm in association with adaptation of modern expertise. The local rice landraces can be used in rice breeding program with available genetic and phenotypic diversity. The phenotypic variation of rice germplasms is expressed by genotypes *per se* and its interaction with environment (Schmid, 1992).

The agro-morphological characterization describes the available phenotypic diversity in the germplasms and assist the potential superior gene restoration and transfer in rice breeding program (Sivakumar *et al*., 2021). And, hence help to accelerate the rice breeding activities to provide better genetic materials (rice variety) to register or release. So, the present experiment was laid to characterize the Nepalese rice landrace for their qualitative agro-morphological distinction.

MATERIALS AND METHODS

Experimental site

The field experiments were conducted on the research farms of Agriculture and Forestry University (AFU) started from July to October, 2020. The experimental site was at 27^0 64'Northern latitude and 84^0 35' Eastern longitude and at an altitude of 229.2 meters above sea level. Composite soil sample was taken from the field after land preparation. The sample was air dried and sent to Soil Science Division (SSD), NARC, Khumaltar, Lalitpur, Nepal. Chemical properties of soil were analyzed in laboratory of SSD. The analyzed result is given in Table 1.

Table 1: Chemical Properties of soil sample from experimental site, Rampur 2020.

Soil pH	Organic Matter (OM)	Total Nitrogen	Available P_2O_5	Available K_2O
5.06	1.21%	0.08 %	42.1 mg/kg	61.3 mg/kg
Very acidic	Low	Medium	High	Medium

Plant materials and Nursery bed

The plant materials were collected from NAGRC, Nepal. It comprised 188 rice accessions (Appendix 1) with 20 grams of seed for each. Dry bed nursery was established on 5th July, 2020 with plot size of 0.25 m^2 (1m × 0.5 m). No Farm Yard Manures and chemical fertilizers were applied on the Nursery bed. The ages of the seedlings were 26 days old at time of transplanting.

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Field layout and Transplanting

The trial was experimented in alpha lattice design with 12 blocks per replication and 16 accessions in each block (4 plots of last blocks in each replication were not used in experiment) replicated twice. The size of each plot was 2 m^2 (1m × 2m) with 20 cm spaced rows and 15 cm spaced hills in every rows. Blocks were spaced with 0.5 m distance and plots were continuous within a block. Replications were also maintained at 0.5 m space. Transplanting of the 2-3 seedlings per hill were done on 31st July.

Data recording and analysis

The observations were recorded for 29 qualitative variables (Table 2) among which there were 12 leaf characters, 6 culm characters, 4 panicle and 7 grain characters. The landraces were characterized using plant descriptors -descriptors for wild and cultivated rice published by Bioversity International, IRRI and WARDA, 2007. MS-Excel was applied for Data entry, computation of Shannon-Weiner diversity index and Minitab-19 for pie diagram.

Table 2: List of 29 qualitative characters with their variables (published by Biodiversity International, IRRI and WARDA, 2007) on rice.

S.N.	Characters	Variables
1	Basal leaf sheath: colour	Green
		Light purple
		Purple
		Green with Purple lines
2	Leaf sheath: Anthocyanin color	Absent
		Weak
		Medium
		Strong
3	Leaf Blade: Intensity of Green color	Dark
		Light
		Medium
4	Leaf blade: pubescence	Glabrous
		Intermediate
		Pubescent
5	Leaf Blade: Anthocyanin colour	Absent
		Present
6	Leaf blade: Distribution of anthocyanin coloration	Absent
		on tips only
		on margin only
		in blotches
		even (uniform purple)
7	Leaf Auricle colour	Absent
		Light purple
		Purple
		Purple lines
		Whitish
		Yellowish green
8	Leaf Collar colour	Absent
		Green
		Light green
		Purple
9	Leaf Ligule shape	Acute
		Acuminate
10	Leaf Ligule colour	Absent
		Light purple
		Purple
		Purple lines

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		Whitish
11	Flag leaf attitude (early observation)	Erect Semi-Erect Horizontal Descending
12	Flag leaf attitude (late observation)	Erect Semi-Erect Horizontal Descending
13	Culm: habit	Erect Intermediate Open Spreading Procumbent
14	Culm: Anthocyanin coloration on nodes	Absent Purple Light purple Purple lines
15	Culm underlying node colour	No colour Light gold Green
16	Culm: internode anthocyanin	Absent Purple Purple lines
17	Culm: Underlying internode coloration	No color Light gold Green
18	Culm: lodging resistance	Very weak Weak Intermediate Strong Very strong
19	Panicle: attitude of main axis	Upright semi-upright Slightly Drooping Strongly Drooping
20	Panicle: attitude of branches	Erect/Compact Semi-erect/semi-compact Spreading/open Horizontal Drooping
21	Panicle: secondary branching	Absent Sparse/light Dense/medium Clustered/heavy
22	Panicle: exertion	Enclosed Partly exerted Just exerted Moderately well exerted Well exerted
23	Awn color (early)	Absent White Straw Gold Brown Light green Red Purple Black
24	Awn distribution	Absent (Awnless)

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		Erect
		Tip
		Upper half
		Upper three quarter only
		Upper quarter
		Whole
25	Lemma Palea: color	White
		White straw
		Gold and gold furrows
		Brown (tawny)
		Brown spots
		Brown furrows
		Purple
		Reddish to light purple
		Purple spots
		Purple furrows
		Black
		Green
		Purple shades on green
		Yellowish green
		Green stripped white
26	Lemma: Apiculus color	Absent
		Brown
		Green
		Purple
		Purple apex
		Red
		Black
		Straw
		White
27	Lemma: anthocyanin coloration of area below apiculus	Absent
		very weak
		Weak
		Medium
		Strong
28	Sterile lemma colour	Straw
		Gold
		Red
		Purple
		Brown
		White
29	Stigma: colour	White
		Light green
		Light purple
		Purple

RESULTS AND DISCUSSION

Qualitative agro-morphologies are considered as indicators in the characterization of rice landraces and are less dependent on environmental influences (Kinoshita *et al*., 2017). The salient features to identify a plant variety are qualitative characters which are mostly under genetic control. Qualitative characters are pivotal for plant description and influenced generally by the consumer's preference, socio-economic scenario and natural selection (Rana *et al*., 2006; Bajracharya *et al*., 2010). The rice landraces collected from NAGRC exhibited surplus variation for 26 qualitative characters among studied 29 characters.

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Leaf characters

Rice landraces were characterized for qualitative leaf traits at late vegetative and flowering stages and variation was observed in the accessions for 10 leaf characters among studied 12 characters. Ten leaf characters were different for trait expression among studied 188 rice accessions (Figure 1) except leaf ligule color and flag leaf attitude (early observation). Basal leaf sheath color unveiled mostly green coloration with 180 accessions (95.74%) along with 4 lines having green coloration with purple lines, 3 light purple and 1 purple pigmentation. Most of the accessions were without leaf sheath anthocyanin color and only 8 accessions were with leaf sheath anthocyanin color (5 with weak and 3 with medium expression). Similarly, 89 accessions exhibited light green leaf blades while 76 accessions revealed medium green and 23 revealed dark green leaf blades for intensity of green color in this experiment. Likewise, only 4 rice accessions showed pubescent leaf blade and most of the accessions (113) expressed glabrous leaf blade while 71 accessions were with intermediate expression. For anthocyanin coloration and its distribution, most of the accessions with 184 lines (97.87%) did not show anthocyanin pigmentation and among 4 pigmented accessions, 3 showed on leaf blade margins only and 1 in blotches. Moreover, 187 accessions showed leaf auricle coloration with majority accessions having yellowish-green auricles while 72 were with whitish auricles and a single accession had light purple leaf auricle. Similarly, rice accessions with 186 lines expressed light green leaf collar color and a rice accession showed green and an accession exhibited purple leaf collar color. All of the rice accession displayed whitish ligule with majority acuminate and few (10) acute ligules shape. Flag leaf attitude did not show any variation in early observation (all erect leaves) but showed different expressions in late observation with 62 descending, 3 horizontal expressions and 8 semi-erect expressions while remaining had the same erect leaves as like in early observations. Thakur (1981) reported leaf angle as erect, horizontal or droopy and was largely influenced by leaf length. The wider the angle, the more the spread of leaves for light interception, especially in the lower leaves (Saitoh *et al*., 2002).

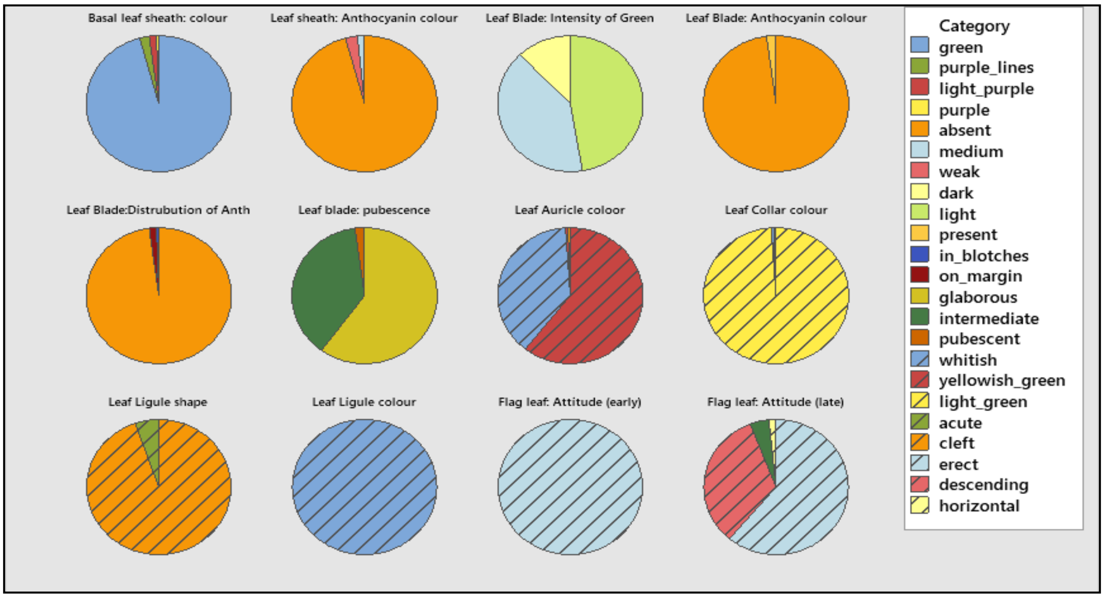

Figure 1: Qualitative leaf characters of 188 rice accessions from NAGRC, 2020.

Culm character

Rice landraces were characterized for culm traits at flowering stages and variation was observed among the accessions for 6 qualitative culm characters. Culm habit was measured as the estimated average angle of inclination of the base of the main culm from vertical after

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flowering. If the average angle is less than 15^0, the trait is called erect. If average angle is about 20^0, the trait is called semi-erect. The trait is called open when average angle is about 40^0 and it is spreading if angle is more than 60^0 and less than 80^0. When culms and its lower parts rests on grounds it is called procumbent. There was high variation for culm habit (Figure 2) with a large number of landraces displaying procumbent and erect culm habit among 188 rice accessions. Culm lodging resistance, scored at maturity based on the observed degree of lodging, also showed high variation among 188 accessions. It was measured in five categories and most of the accessions showed very weak lodging resistance (96 accessions) followed by accessions with strong culm (74 accessions) and weak culm lodging resistance (18 rice accessions).

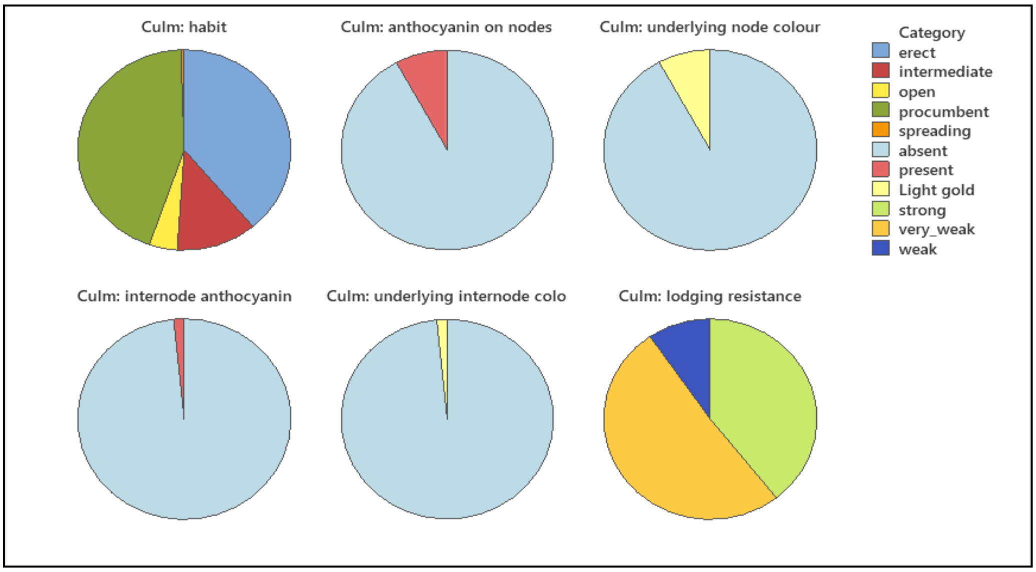

Figure 2: Qualitative culm characters of 188 rice accessions from NAGRC, 2020

Among 188 rice accessions, the presence and distribution of purple color, observed on the outer surface of nodes and internodes of culm at flowering to near maturity, exhibited high variation. Majority of the accessions didn't show any anthocyanin on nodes and internodes. Only 15 accessions on the nodes of the culm exhibited anthocyanin coloration and three accessions showed on internodes.

Panicle characters

Panicles, the top part of the rice plant, form the rice inflorescence. It is carried on the last inter-node (Yoshida & Nagato, 2011). Rice landraces were characterized for panicle traits at near maturity stages and variation was observed in the experiments among the accessions for 4 qualitative panicle characters. The panicle traits were highly varied for 188 rice accessions (Figure 3). The panicle studied for the main axis showed a slightly drooping attitude for the majority of accessions (166) followed by semi-upright (20 accessions) and upright (2 accessions). The attitude of branching showed semi-compact branches on panicles for 143 accessions followed by open (spreading) branches on 33 accessions and compact branches on 12 accessions. Most of the rice accessions (121) exhibited sparse/light secondary (2^0) branching on panicles followed by medium (dense) 2^0 branching on 22 accessions and clustered (heavy) 2^0 branching on 20 accessions while 25 accessions did not show 2^0 branching on panicles. Studying traits for panicle exertion on 188 accessions, 152 accessions had just exerted panicles and 34 accessions with well exerted panicles. There were two accessions with enclosed panicles on flag leaf.

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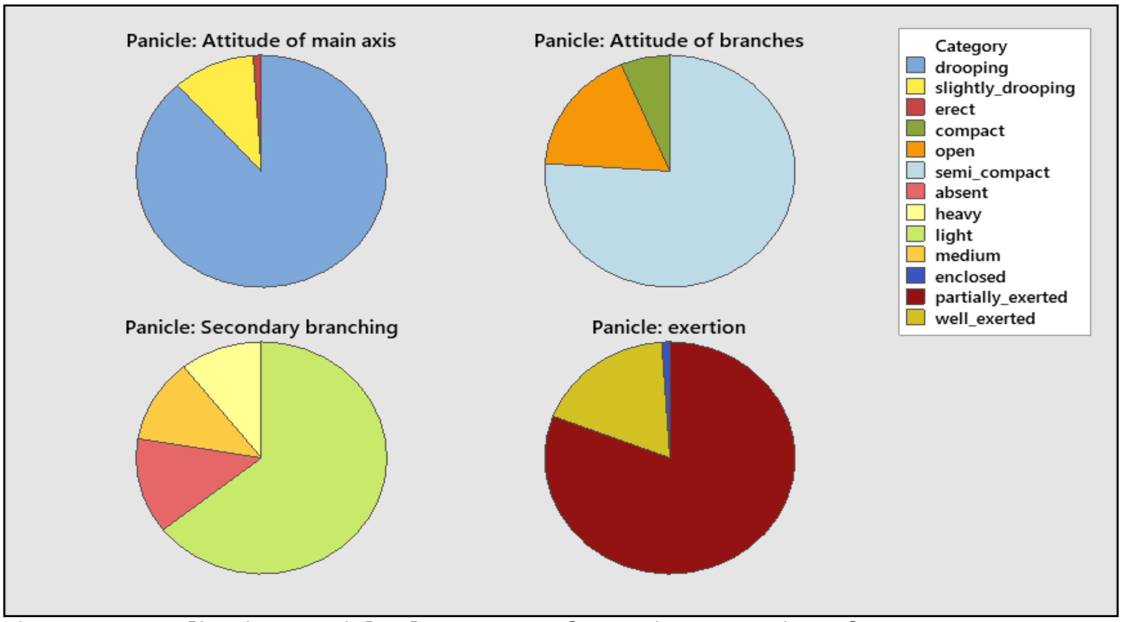

Figure 3: Qualitative panicle characters of 188 rice accessions from NAGRC, 2020

Grains characters

Rice landraces were characterized for grain traits at reproductive stage before harvesting and observed surplus variations among the accessions for grain characters except for stigma colour. The presence of awns is considered an important trait in rice domestication (Hussain *et al*., 2014). Grains of wild rice have long awns that protect the grains from animal pilfering. The varieties with long awns or strong awns are more resistant to bird attack than the varieties with no awns. On the other hand, cultivated rice varieties have short awns allowing for easier harvesting than varieties with long awns (Hussain *et al*., 2014).

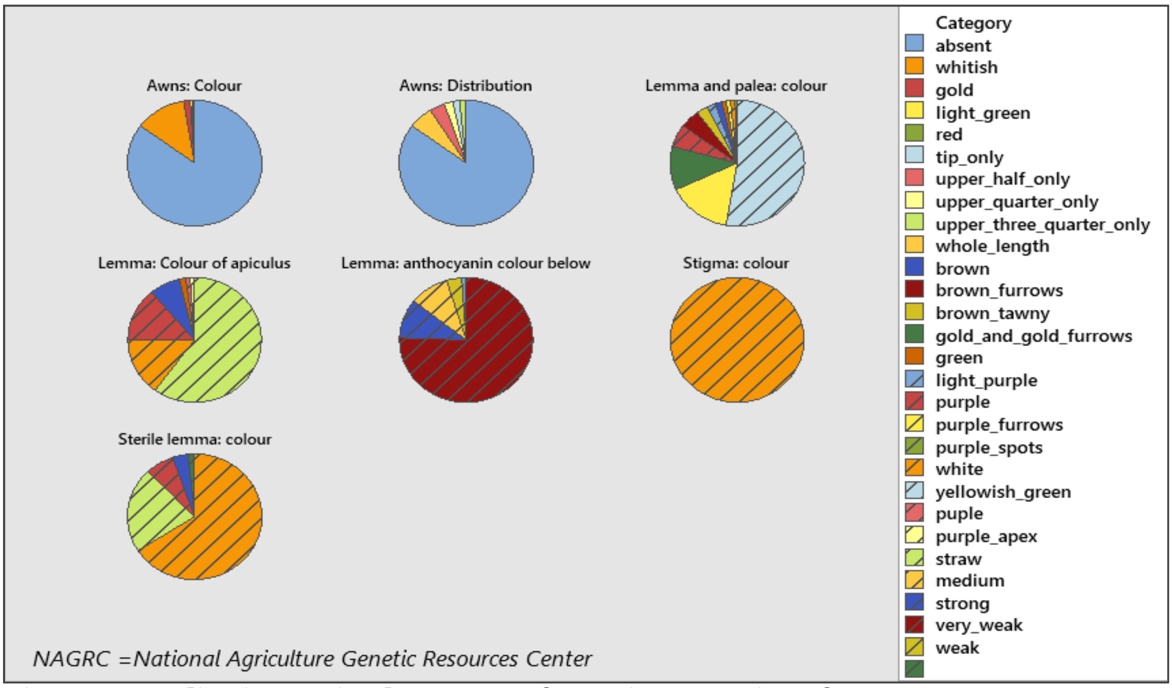

Figure 4:Qualitative grain characters of 188 rice accessions from NAGRC, 2020

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The rice accessions exhibited variation for grain qualitative characters (Figure 4). Among 188 accessions, 160 were awnless accessions. The coloration of awn was also varying for 28 accessions among which 23 were white, 3 (NGRC 03423, NGRC 06632 and NGRC 07064) had gold and NGRC 06633 had red and NGRC07916 had light green color awn. The distribution of awn among 28 awned accessions, there were 11 accessions with awn throughout the panicle (whole), 3 accessions with awn on upper three quarters of panicles only, 7 accessions with panicles on upper half of the panicle, 4 accessions with awn on upper quarters of panicle only and 3 accessions with awn on tip of the panicles only.The rice accessions were found with apiculus on lemma showing high variation for its color. Most of the accessions (113) exhibited straw colored apiculus followed by 30 accessions with purple colored apiculus among which 2 accessions had purple color in apex only and white colored apiculus for 28 accessions. Then, 14 accessions had brown apiculus followed by 3 green colored apiculus on lemma.

Likewise, 186 accessions revealed anthocyanin color of area below apiculus on lemma. Among these accessions, 142 accessions had very weak expression, 7 accessions with weak anthocyanin expression, 18 accessions with medium and 19 accessions with strong expression.The lemma palea coloration showed surplus variation in 188 accessions. Most of the accessions (99) exhibited yellowish green lemma palea followed by 31 green colored (lemma palea) accessions and 21 gold and gold furrows (lemma palea color) accessions. Likewise, 18 accessions revealed purple lemma palea (11 full purple; 4 reddish to light purple; 2 purple furrows and 1purple spots), 17 accessions had brown colored lemma palea (9 with brown furrows; 8 with brown/tawny) and 2 white lemma palea colored accessions. All of the accessions exhibited white colored stigma seen under the lens. The sterile lemma color was varied for 188 accessions among which 124 accessions had white colored sterile lemma followed by 41 accessions with straw colored sterile lemma. The purple-colored sterile lemma was found in 13 accessions and brown-colored in 7 accessions.

Diversity Indices

The Shannon–Wiener index, a well-known diversity index developed by Claude E. Shannon and Norbert Wiener in 1949 (Spellerberg & Fedor, 2003), explains the state of diversity in a particular attribute and species. The higher the value of the diversity index, the more diversity present in the trait (Joshi, 2021). The diversity index (H') computed from 26 qualitative characters exhibited low index value (H' =0.1) for distribution of anthocyanin colour for leaf blade and high index value (H'=0.89) for intensity of green colour of leaf blade (Table 3). It explains the prospect of experimenting landrace to advance rice breeding (Demeke *et al*., 2022). The Shannon-Weiner diversity index (H') values were highest for intensity of green colour of leaf blade followed by culm lodging resistance (0.85), secondary branching of panicle (0.75), culm habit (0.71), leaf blade pubescence (0.69), lemma palea colour (0.67), lemma apiculus colour (0.66) and sterile lemma colour (0.66). Bajracharya *et al*. (2006) and Poudel *et al*. (2020) also found similar results regarding the state of diversity among the landraces. These qualitative characters are the source of different allelic sets that help breeders to excel rice improvement research.

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Table 3: Shannon-Weiner diversity index (H′) for 26 qualitative characters in 188 rice landraces.

S.N.	Characters	Shannon-Weiner Index (H′)
1	Basal leaf sheath: colour	0.16
2	Leaf sheath: Anthocyanin color	0.19
3	Leaf Blade: Intensity of Green color	0.89
4	Leaf blade: pubescence	0.69
5	Leaf Blade: Anthocyanin colour	0.15
6	Leaf blade: Distribution of anthocyanin coloration	0.10
7	Leaf Auricle colour	0.52
8	Leaf Collar colour	0.06
9	Leaf Ligule shape	0.30
10	Flag leaf attitude (late observation)	0.63
11	Culm: habit	0.71
12	Culm: Anthocyanin coloration on nodes	0.40
13	Culm underlying node colour	0.37
14	Culm: internode anthocyanin	0.12
15	Culm: Underlying internode coloration	0.12
16	Culm: lodging resistance	0.85
17	Panicle: attitude of main axis	0.36
18	Panicle: attitude of branches	0.63
19	Panicle: secondary branching	0.75
20	Panicle: exertion	0.48
21	Awn color (early)	0.32
22	Awn distribution	0.36
23	Lemma Palea: color	0.67
24	Lemma: Apiculus color	0.66
25	Lemma: anthocyanin coloration of area below apiculus	0.52
26	Sterile lemma colour	0.66

CONCLUSION

Characterization of 188 rice landraces unveiled variation in 26 qualitative characters among studied 29 qualitative characters. White colour leaf ligule and erect flag leaf attitude at early observation and white stigma colour were expressed in all rice landraces. All other 26 qualitative characters revealed diverse expressions of different traits for each character. Lemma and palea colour (10 traits among 15), and colour of apiculus of lemma (6 traits among 9) showed maximum number of qualitative traits. The intensity of green colour of leaf blade, culm lodging resistance and culm habit, secondary branching of panicle, and lemma and palea colour, lemma apiculus colour and sterile lemma colour explained the greater trait diversity in studied characters. The expressed variation in qualitative characters approves the presence of abundant genetic diversity in the collection and that ultimately signifies the effective utility of local collection in rice breeding programs.

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Authors' Contributions

R. Kharel, S. Subedi, D. Ghimire, and S. Shrestha designed and performed the experiment, recorded and analyzed data and wrote the manuscript.

Conflict of Interest

The authors of the paper declare that there is no conflict of interest for the publication of this manuscript.

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