

## Research Article

# Identification of micronutrient-rich germplasms of barley for mid-hills and Terai region of Nepal

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## ABSTRACT

Zinc and iron deficiencies, common in many parts of the world, having risk factors that raise the burden of disease with high child and adult mortality. Breeding efforts for exploring barley grains with higher zinc and iron are underway. A study was conducted during the normal barley growing season of 2017 to identify the barley genotypes with higher concentration of protein, starch, iron and zinc for the mid-hill and Terai conditions of Nepal. An augmented block design was followed, and two improved varieties i.e., Bonus and Solu Uwa were used as repeated checks. The average grain protein, starch, zinc, and iron content were 11.9%, 55.6%, 47.4 mg/kg and 41.4 mg/kg at Dailekh and 11.3%, 53.3%, 46.6 mg/kg and 39.9 mg/kg at Rampur, respectively. The grain protein content was found up to 19.8% (AM-55, AM-45, AM-88 at Dailekh) and 16.6% (AM-81, AM-77, AM-98 at Rampur). Similarly, the highest grain zinc content was found 97 mg/kg (AM-6, AM-138 and AM-125 at Dailekh) and 92 mg/kg (AM-226, AM-99 and AM-227 mg/kg at Rampur). The highest iron content 71 mg/kg (AM-2, AM-43, AM-4 at Dailekh) and 63 mg/kg (AM-90, AM-168 and AM-92 at Rampur). The identified genotypes with higher grain elements concentration are valuable resources for Nepal's barley breeding program that helps develop quality and nutritious barley varieties.

**Keywords:** Barley, iron, protein, starch zinc

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## INTRODUCTION

Barley is a staple food crop in many parts of the world, including the Andean nations, the Baltic States, North, and East Africa, the highlands of Asia, and North and East Africa (El-Haramein & Grando, 2010). Following rice, wheat, and maize, the FAO's ranking of major cereals by output and production area is barley (Newman & Newman, 2006). In Nepal, barley is traditionally grown in small areas throughout the country; as a food crop, it is common in the Himalayan highlands, especially in the western region where the production environments are harsh, and food security is a major challenge (Pokharel *et al.*, 2021).

Barley grain contains around 65–68% carbohydrates, 10–17% proteins, 4–9% free lipids, and 1.5–2.5% minerals, respectively (Quinde *et al.*, 2004). Because of the crop's wider

adaptability and resilient nature and recent findings of the health benefits of barley grains as well, there is a renewed importance as a human food (Newton *et al.*, 2011; Idehen *et al.*, 2017). Further, due to the presence of genes involved in the mobilization and uptake of Fe and Zn, barley is less sensitive to low levels of Fe and Zn in soil. Lacking this capability, other large-scale crops, like rice, are significantly impacted by low levels of Fe and Zn in the soil in terms of mineral uptake in the grains (Nakanishi *et al.*, 2000; Takahashi *et al.*, 1999). In addition to other genes, HvNAS1, HvNAAT-A, HvNAAT-B, HvDMAS1, IDS2, and IDS3 are up-regulated in Fe-deficient Barley Roots (Nakanishi *et al.*, 2000; Takahashi *et al.*, 1999).

As the initial stage in improving grain quality and biofortifying these components in barley, it is possible to examine the state of these attributes in the kernel by employing a variety of barley germplasm. One of the main objectives of the national barley breeding program in Nepal is maintenance and improvement of barley genetic resources for food, feed, and fodder in common and marginal habitats, mainly for abiotic stress circumstances. However, there are only limited studies focused on barley in Nepal. Thus in current paper we attempted to find out the contents of starch, protein, Zn, and Fe in barley grain under optimal production environments two different agro ecological situation of mid hills, represented by Dailekh and Tarai represented by Chitwan, Rampur.

## MATERIALS AND METHODS

### Barley genotypes

Three hundred forty barley genotypes were obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA), and two checks (Bonus and Solu-Uwa) from Nepal were evaluated. The Nepalese barley varieties Bonus (two-rowed, hulled type) and Solu-Uwa (six-rowed, hull-less type) were developed and released for general cultivation in 1972 and 1992, respectively (Baniya *et al.*, 1997). Among the tested genotypes, 230 were from the low-input breeding program and 86 were from the high-input breeding program, and the other 24 genotypes were of mixed type. Furthermore, 280 were hulled types, 60 were hull-less types, 138 were two-row, and 202 were six-row types. Likewise, about 74% were advanced breeding lines, 9.5% were Gene-bank accessions, and 16.5% were barley varieties released by different breeding programs (India, Australia, USA, Canada, Morocco, and Nepal). A detailed description of AM-2014 can be found in Amezrou *et al.* (2017).

### Field experiments

Two field studies were conducted at mid hills of Dailekh district and Tarai areas of Rampur (Chitwan district) of Nepal. The research field of Agriculture Research Station, Kimugaun Dailekh, and the National Maize Research Program, Rampur, Chitawan, Nepal, were used to test the genotypes. Rampur is located at 27.40 N, 84.20 E, and 228 masl, and Dailekh is located at an altitude of 1355 m above mean sea level with 28.8479' North latitude and 81.7225' East longitude. Rampur had a warm climate, while Dailekh had a cool climate for producing barley. The experiment was set up in an augmented block design with two repeated checks (Bonus and Solu Uwa) at both locations. Each block included 20 entries, making a total of 17 blocks. At Rampur and Dailekh, respectively, planting was done on December 8 and November 12, 2017. Each genotype was seeded on a 1.25 m<sup>2</sup> area (two rows of 2.5 m length). Continuous seeding at a rate of 80 kg/ha was done with a row-to-row spacing of 25 cm. Chemical fertilizers was applied at 80 kg N, 40 kg P<sub>2</sub>O<sub>5</sub>, and 30 K<sub>2</sub>O kg/ha. At sowing, 1/3 of N (urea), full dose of K<sub>2</sub>O (muriate of potash) and P<sub>2</sub>O<sub>5</sub>

(diammonium phosphate) were given. Remaining N (Urea) was applied two times, at the crown root initiation and booting stages. As and when necessary, further recommended crop management procedures were performed.

### Soil properties of the experimental blocks

Soil samples from each experimental field of both locations were collected before sowing the barley grain. The collected soil samples were analyzed at the laboratory of the National Soil Science Research Centre, Khumaltar, Lalitpur, Nepal. The soil analysis result of each site revealed that the soil pH was slightly higher at Rampur than Dailekh (Table 1). The available potassium and phosphorous were higher at Dailekh than at Rampur. The concentration of iron and zinc were almost similar at both locations. The soil type was sandy at Rampur, but in contrast, it was loamy at Dailekh (Table 1).

**Table 1. Soil properties of the experimental sites**

Parameters	Dailekh	Rampur
pH	5.13	6
OM%	2.94	3.33
Total N%	0.12	0.14
Available P <sub>2</sub> O <sub>5</sub> , mg/kg	32.7	18.4
Available K <sub>2</sub> O, mg/kg	116.2	59.4
Available Fe, mg/kg	41.6	48.8
Available Zn, mg/kg	0.54	0.53
Sand, %	41	62.4
Silt, %	44.5	28.4
Clay, %	14.5	9.2
Class	Loam	Sandy Loam

Source: National Soil Science Research Center, Khumaltar, Lalitpur

### Barley grain sample collection and laboratory analysis

Five randomly chosen spikes from each barley genotype were manually collected at maturity, stored in designated envelopes, and sun-dried for two days to prepare for the grain element analysis. Grain was manually threshed, cleaned, and kept in paper sacks to remove any metal contamination (contact with any metal was avoided).

To analyze the grain protein, starch, Fe, and Zn concentration, the collected samples from both locations were sent to Morocco's Institut National de la Recherche Agronomique-Maroc (INRA-Maroc). Sample preparation and analysis methods were completed at INRA-Maroc following Miller and Rutzke (2003) for the Fe and Zn tests. Grain samples were dried in an oven for 16 hours at 105 °C, overnight at 200 °C, and then for 16 hours at 450 °C.

According to the technique established by Health Canada (1985), the leftover ash was then digested in 4 ml of nitric acid to dissolve the organic content and 5 ml of diluted hydrochloric acid (9.25%) to dissolve the remaining inorganic residue. Black checks and internationally acknowledged reference materials were utilized to uphold the analytical quality assurance. Near-infrared reflectance spectroscopy (NIRS), a near-infrared analyzer by transmission model "Infraneo" from Chopin, was used to determine the protein content of approximately 10 g of cleaned barley grain samples following AACCI Method 39-25.01. Before being scanned using a Foss Infratec 1241 at the ICARDA quality laboratory in compliance with the steps and AACCI Method 76-31.01, the barley samples were first ground using a UDY cyclone to a thickness of 1 mil.

### Statistical analysis of the data

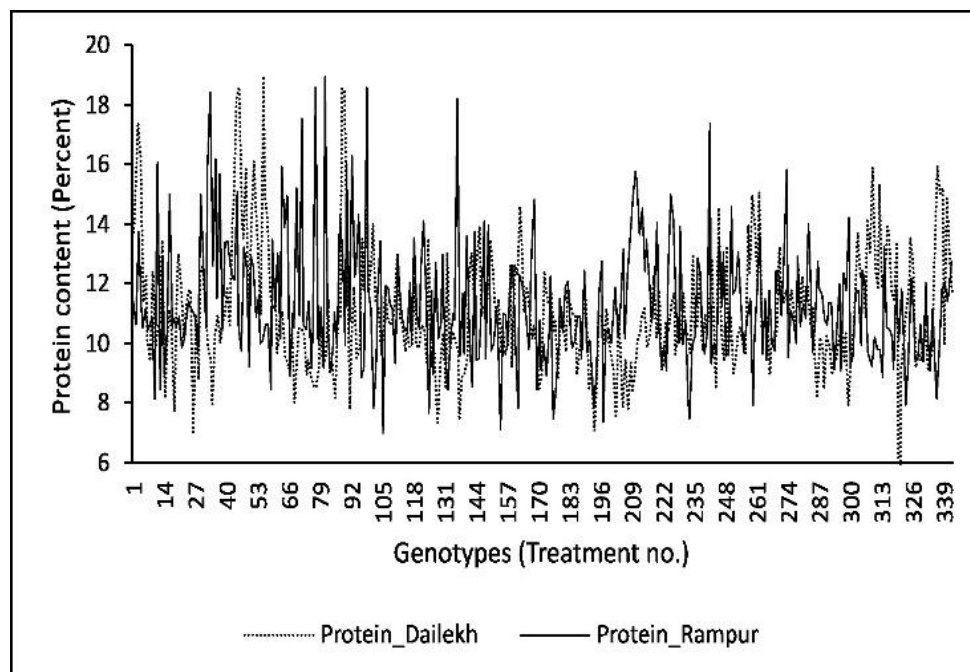
The laboratory data were used for statistical analysis. Microsoft Excel was used for data processing and descriptive statistical analysis. ACBD-R statistical software (Rodriguez *et al.*, 2016) was used for mean separation of protein, starch, zinc, and iron concentration.

## RESULTS AND DISCUSSION

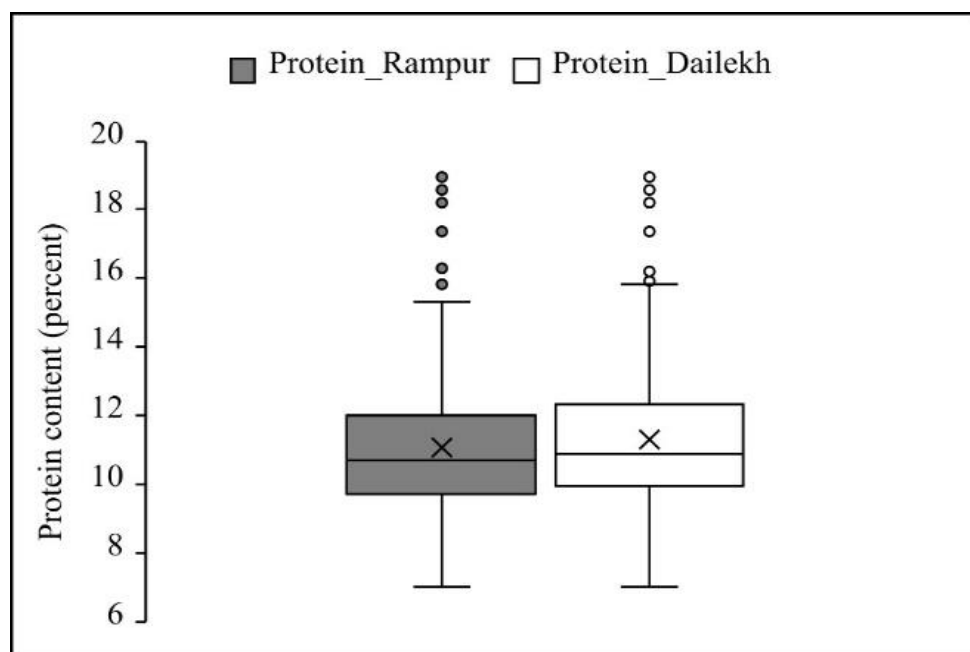
Fifty genotypes from Dailekh and 53 genotypes from Rampur genotypes had higher iron concentrations (more than 60 mg/kg of barley grain). Likewise, 80 genotypes at Dailekh and 84 at Rampur were detected with higher zinc concentrations (more than 45 mg/ kg of barley grain). A detail of the research result is presented separately as follows.

### Protein and starch content

About 17% of genotypes (n=58 at Dailekh and 62 at Rampur) were detected as having higher protein content (more than 13% grain protein content). The boxplot of protein contents at Dailekh and Rampur has been presented in Figure 1a. The average protein contents of barley grains were 11.9 and 11.3 % at Dailekh and Rampur, respectively (Figure 1b). The average grain protein of the selected genotypes was slightly higher at Dailekh than at Rampur (Figure 1b). The grain protein ranged from 7.1-19.8% and 6.7-16.6% at Dailekh and Rampur, respectively. The top 20 genotypes with higher protein content at Dailekh were AM-55, AM-45, AM-88, AM-89, AM-44, AM-3, AM-4, AM-2, AM-51, AM-48, AM-309, AM-336, AM-312, AM-338, AM-262, AM-56, AM-259, AM-337, AM-98, AM-340. Likewise, the top 20 genotypes at Rampur were AM-81, AM-77, AM-98, AM-33, AM-136, AM-71, AM-241, AM-92, AM-35, AM-11, AM-90, AM-63, AM-32, AM-273, AM-210, AM-37, AM-211, AM-69, AM-44 and AM-225 (Figure 1a and Figure 1b).

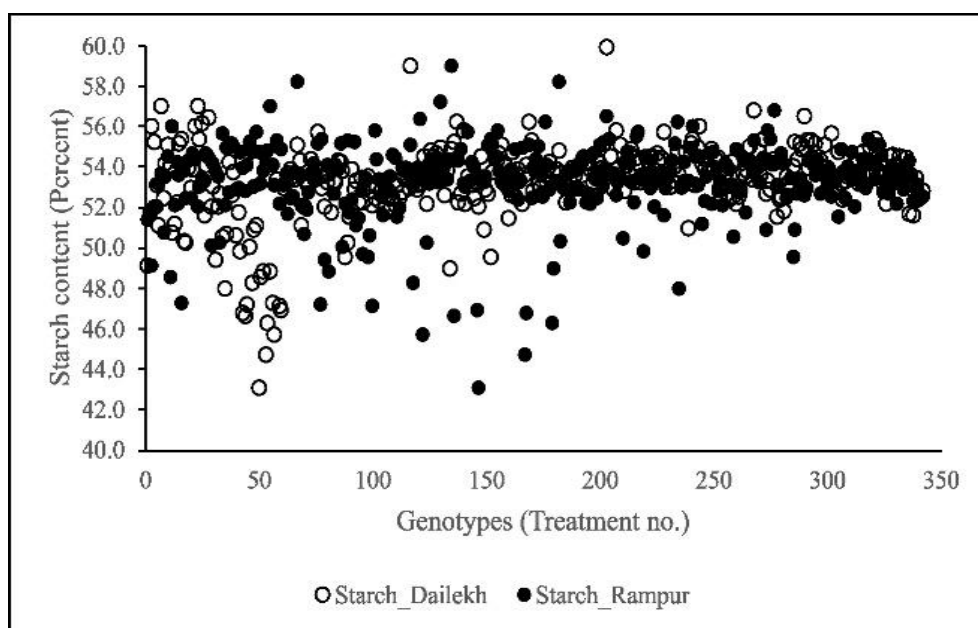


**Figure 1a.** Line graph showing the protein content (%) of barley genotypes cultivated at Dailekh and Rampur



**Figure 1b.** Box plot with error bars showing the protein content (%) of barley genotypes cultivated at Dailekh and Rampur

Likewise, 43 genotypes at Dailekh and 45 at Rampur had more than 55% starch concentration in the barley grain. The average starch contents of barley grains were 55.6% and 53.3 % at Dailekh and Rampur, respectively (Figure 2). The genotypes with higher starch concentration at Dailekh were AM-203, AM-117, AM-7, AM-23, AM-268, AM-28, AM-290, AM-137, AM-25, AM-169, AM-3, AM-22, AM-244, AM-76, AM-140, AM-207, AM-228, AM-302, AM-16, AM-24. Similarly, genotypes AM-135, AM-67, AM-182, AM-130, AM-55, AM-277, AM-203, AM-121, AM-176, AM-234, AM-241, AM-12, AM-49, AM-101, AM-142, AM-155, AM-217, AM-274, AM-34 and AM-216 (Figure 2).

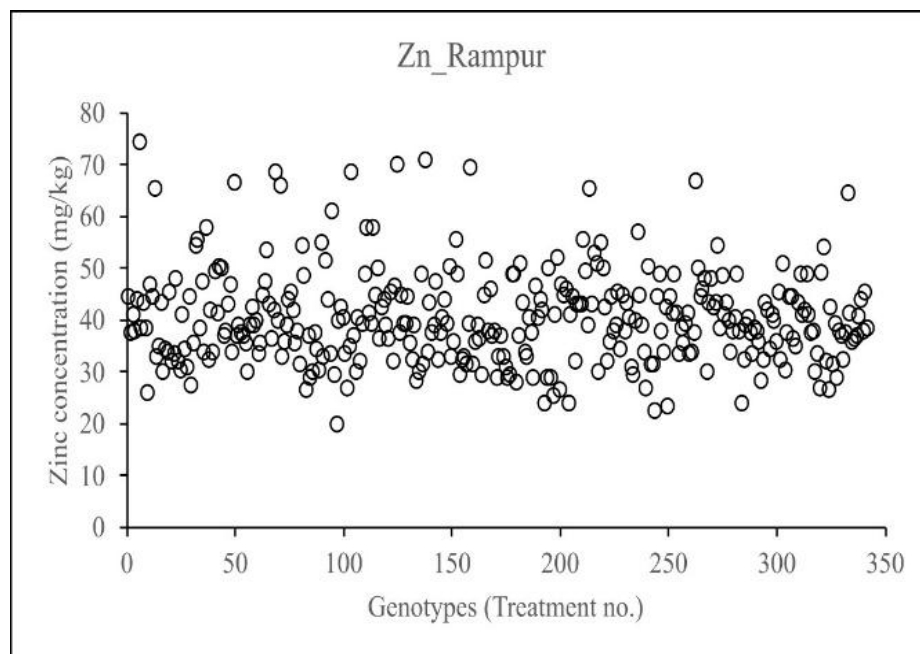


**Figure 2.** Dot graph showing the starch content (%) of barley genotypes cultivated at Dailekh and Rampur

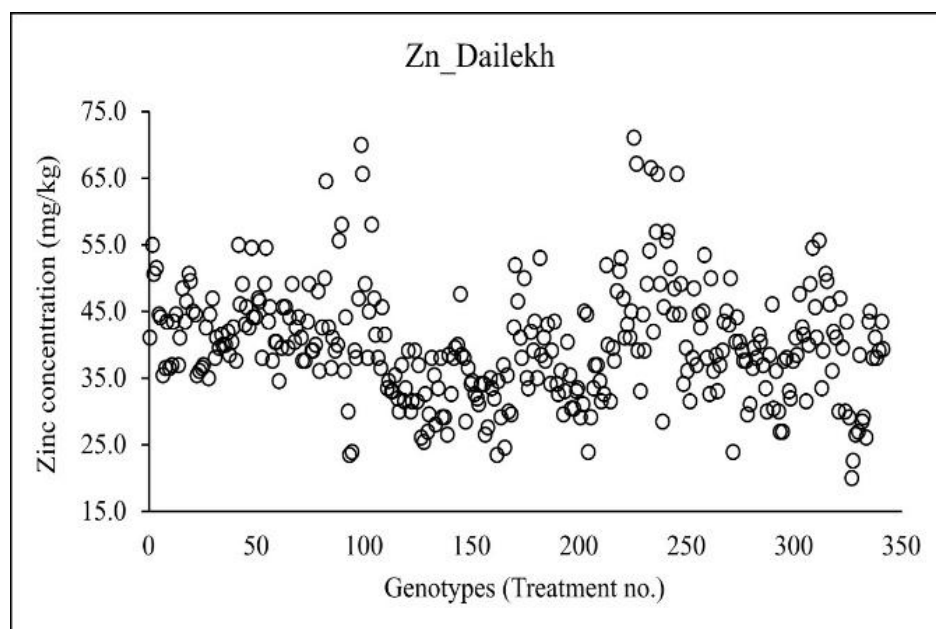


### Barley grain zinc (Zn) concentration

The zinc concentration of the tested genotypes ranged from 20-71 mg/kg at Dailekh and 15-63 mg/kg at Rampur, respectively (Figure 3 and Figure 4). The 80 genotypes at Dailekh and 84 genotypes at Rampur were identified as higher zinc content genotypes.



**Figure 3. Dot graph showing the zinc concentration (mg /kg) of barley genotypes cultivated at Rampur**



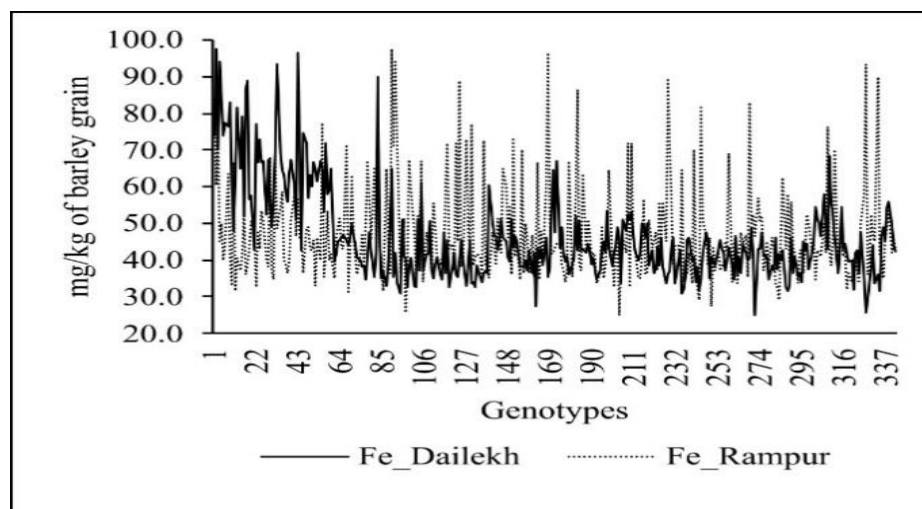
**Figure 4. Dot graph showing the Zinc concentration (mg/kg) of barley genotypes cultivated at Dailekh**

The genotypes AM-6, AM-138, AM-125, AM-159, AM-69, AM-104, AM-263, AM-50, AM-71, AM-13, AM-214, AM-333, AM-95, AM-37, AM-111, AM-114, AM-236, AM-33, AM-152, AM-211, AM-90, AM-219, AM-32, AM-81, AM-273 at Rampur and AM-226, AM-99, AM-227, AM-234, AM-100, AM-237, AM-246, AM-83, AM-104, AM-90, AM-

236, AM-242, AM-241, AM-312, AM-89, AM-2, AM-42, AM-309, AM-48, AM-55, AM-233, AM-259, AM-182, AM-220 and AM-171 were detected as the 25 higher zinc concentration at Dailekh, respectively (Figure 3 and Figure 4).

### Grain iron concentration

Fifty-one genotypes at Dailekh and 53 at Rampur were detected with higher iron-containing genotypes in the barley grain. The average iron concentration at Dailekh and Rampur were 47.4 and 46.6 mg/kg, respectively.



**Figure 5. Line graph showing the iron content (mg/kg) of barley genotypes cultivated at Rampur and Dailekh**

The iron concentration ranged from 24.5-97 mg/kg at Dailekh and 16-92 mg/kg at Rampur. Genotypes AM-2, AM-43, AM-4, AM-33, AM-83, AM-18, AM-17, AM-5, AM-9, AM-13, AM-15, AM-7, AM-22, AM-8, AM-46, AM-1, AM-6, AM-24, AM-44, AM-47, AM-57, AM-3, AM-32, AM-34 and AM-309 at Dailekh and the genotypes AM-90, AM-168, AM-92, AM-327, AM-333, AM-228, AM-124, AM-183, AM-1, AM-269, AM-245, AM-55, AM-130, AM-308, AM-3, AM-93, AM-127, AM-151, AM-136, AM-118, AM-122, AM-208, AM-210, AM-67 and AM-91 at Rampur were among the top 25 genotypes with higher iron concentrations (Figure 5).

In present study, we identified the barley genotypes containing higher concentrations of protein, starch, iron, and zinc in the barley grains. The typical protein, starch, zinc, and iron concentrations in our study are consistent with those in earlier studies. However, at Dailekh and Rampur, the grain protein content ranged from 7.1-19.8% and 6.5-16.6% (Figure 1a, 1b, 2, 3). At Dailekh and Rampur, the starch contents were 43-61.9% and 39-59%, respectively (Figure 2). Protein and starch contents generally showed more variance at Dailekh and Rampur, respectively (Figure 1a, 2). Most of the barley genotypes included in this study were chosen for their ability to thrive in dryland environments with little access to moisture and soil nutrients through ICARDA's low-input barley breeding programs. These genotypes were tried in Morocco at very low temperatures compared to Dailekh and Rampur.

The genotypes with higher protein (AM-55, AM-45 and AM-88 at Dailekh and AM-81, AM-77 and AM-98 at Rampur), higher starch (AM- AM- and AM- at Dailekh and AM- AM- and AM- at Rampur), higher zinc (AM- AM- and AM- at Dailekh and AM- AM- and AM- at Rampur) and higher iron content (AM- AM- and AM- at Dailekh and AM- AM- and AM- at Rampur) content were identified (Fig 1a, 1b, 2, 3, 4).

All genotype used in Nepal conditions were poorly adapted to our environments. The samples of barley grain used in this investigation came from two different geographies and growing environments in Nepal. In contrast to Rampur, where grain was collected, Dailekh depicts a mid-hills climate, whereas Rampur represents a warm environment. There was greater protein and starch levels heterogeneity due to the low thousand kernel weight (TKW) in some genotypes at both locations.

However, the average grain protein, starch, zinc, and iron concentration was higher at Dailekh than at Rampur (Figure 1a, 2, 4, 5) due to the longer duration of crop maturity at Dailekh, where barley genotypes perform better grain assimilation, as opposed to Rampur, where shorter growing seasons result in generally poor grain assimilation in the grain. Using the same genotypes, one location's barley grain had greater Fe and Zn concentrations than the Jemma Shaim station in Morocco (Gyawali *et al.*, 2017).

At Dailekh and Rampur, respectively, genotypes with greater protein content (more than 13%) were found in numbers 58 and 62 (Figure 1a). At Dailekh and Rampur, 51 and 53 genotypes were found to have greater iron concentrations (more than 60 mg/kg of barley grain). Additionally, increased zinc concentrations were found in 84 genotypes at Rampur and 80 genotypes at Dailekh. There are several uses for barley grain, including uses in food, feed, malt, and nutraceuticals. One of the healthiest foods, barley is abundant in energy and contains many minerals (Cavallero *et al.*, 2002; Quinde *et al.*, 2004). According to recent genomic studies, the barley genome has numerous genes involved in the uptake and mobilization of minerals, particularly Fe and Zn, which are absent in other key food crops like rice (Nakanishi *et al.*, 2000; Takahashi *et al.*, 1999). The genotypes from ICARDA's breeding efforts for barley provide crucial resources for high-gain quality features, particularly increased mineral contents, protein, and soluble fibres like -Glucan. Gyawali *et al.* (2017) discovered numerous QTLs for 13 mineral nutrients, including Fe and Zn, in the barley genome.

## CONCLUSION

The genotypes with higher concentrations of protein, starch, iron, and zinc found in this study are crucial for Nepal's barley breeding program's goal of improving quality attributes. The Nepali barley breeders have better opportunities to access the genotypes used in this study to diversify their crossing blocks and include adapted genotypes into their crossing programs. This will widen the opportunities for molecular breeders, and the SNPs connected to the QTL may be useful in the next steps of molecular barley breeding initiatives.

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### Authors' Contributions

BBP – prepared the research proposal, carried out field experimentation, data collection, data analysis, interpretation of the results, and manuscript preparation; PA, ST, and ST helped in field experimentation and collecting phenotypic data and barley spike collection from the research field of National Maize Research Program, Rampur, Chitwan and Agriculture Research Station Dailekh. All the authors have read and approved the final manuscript.

### Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

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