

# Original Research Article

## Performance of Biofortified Spring Wheat Genotypes for Grain Zinc and Iron Concentration, Grain Yield and Associated Traits in Terai/plains of Nepal

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### ABSTRACT

Breeding for nutrient-rich high yielding wheat varieties is one of the most economical and feasible ways to improve micronutrient deficiency and building better consumer health among the rural people of South Asia. To identify the Zinc (Zn) and Iron (Fe) enriched high yielding wheat genotypes, 7th Harvest Plus Yield Trial (7th HPYT) and 8th Harvest Plus Yield Trial (8th HPYT) both composed of 50 genotypes (including two CIMMYT checks "Kachu#1 and Baj#1 and one Local Check "Gautam") were evaluated in alpha lattice design with 2 replications under timely sown irrigated condition at NWRP, Bhairahawa during 2016/17 and 2017/18. The grain Zn concentration and Fe concentration varies among genotypes from 23.8 to 42.4 ppm and 20.6 to 60.6 ppm, respectively. The highly significant positive correlation was found between grain zinc and iron concentration ( $r = 0.74^{**}$  in 7th HPYT and  $r=0.67^{**}$  in 8th HPYT). This highly positive significant relation between grain Zn and grain Fe indicates that it is feasible to simultaneously improve both micronutrients. In addition, this study reveals that thousand grains weight (TGW) has non-significant negative ( $r = -0.1$  and  $r=-0.1$  in 7th HPYT) to highly significant positive correlation ( $r = 0.3$  and  $r=0.4$  in 7th HPYT) with grain zinc and iron concentrations. As Nepali farmers major trait of interest after grain yield is TGW, this showed that Zn and Fe enriched varieties has higher TGW. The 9 genotypes in 7th HPYT and 48 genotypes in 8th HPYT, showed higher grain yield than local check variety "GAUTAM" which indicates that bio-fortified genotypes are capable of producing higher grain yield with added micronutrient supplements in them. This study recommended 17 genotypes from the 7th HPYT and 38 genotypes from the 8thHPYT based on higher grain yield, grain Zn and Fe concentration and these lines were included in national yield trial for further evaluation in different agro-ecologies. The genotypes with higher grain Zn and Fe concentration viz., 7HPYT409, 7HPYT410, 8HPYT417, 8HPYT404 and 7HPYT442 could be used as donor parents in national wheat breeding program and high yielding genotypes 7HPYT448, 7HPYT418, 7HPYT426, 7HPYT413, 8HPYT415, 8HPYT431, 8HPYT429, 8HPYT407 and 8HPYT405 would be further evaluated throughout the terai region of Nepal, and outstanding genotype could be released as variety for terai/plains of Nepal.

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*Keywords: Biofortification, grain Zn and Fe concentration, grain yield, wheat*

## 1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is a globally grown cereal crop with an annual estimated production of around 785 million MT in 2023/2024 and is grown on around 220.7 million hectares worldwide (FAOSTAT, 2023). Wheat is consumed by 35% of the world's population and contributes 20% of human calories and protein across the world [1]. In the context of Nepal, wheat ranks third after rice (*Oryza sativa* L.) and Maize (*Zea mays* L.) in production but ranks second in consumption after rice. During 2021/2022, the area under cultivation & production were 716978 ha and 2144568 metric tons respectively [2]. More than half (55%) of the wheat area is in Terai which contributes 62% of total production [2].

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According to USAID, around 1 million (36%) of children below 5 years of age are suffering from chronic malnutrition (stunting or low height-for-age) while around 10% suffer from acute malnutrition (low weight-for-height) creating an alarming situation for Government of Nepal [3]. To reduce malnutrition Calderini & Ortiz Monasterio (2003) identified that developing more nutrient-dense staple food crops could be a possible option [4]. The Harvest Plus initiative, a program launched by CGIAR (Consultative Group on International Agricultural Research) focusing on breeding and dissemination of food crops with high micronutrient contents [5]. The Harvest Plus project works with different national and international partners to eliminate micronutrient deficiencies like Zn and Fe through the fortification of staple food crops. The deficiency of Fe and Zn in dietary content affects more than two billion people globally [6]. The global average prevalence of Zn deficiency has been estimated to be 31% with the most severe expression in Africa and South Asia. Its deficiency is known to significantly increase the risk of many diseases viz., diarrhea, pneumonia and malaria, therefore Zn deficiency has been linked to the morbidity and mortality of children. The resulting deficiency of Fe and Zn in developing countries is primarily associated with consumption of cereal-based foods with low concentration and reduced bioavailability of Zn and Fe [7]. This Harvest Plus project improves the nutrient content by supplementing essential minerals and vitamins and this process is considered as one of the most economical and effective solution to human micronutrient deficiency issues [8].

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The biofortified wheat varieties developed in Harvest Plus project are 20-30% better in Zn & Fe concentration (grain) than the best local checks of Nepal. They are also agronomically superior (around 5%) and capable of escaping (due to earliness) or tolerating the terminal heat stress. The Zn and Fe concentration in biofortified wheat genotypes range from 19-52 mg kg<sup>-1</sup> and 23-52 mg kg<sup>-1</sup>, respectively [9]. In South Asia, nutritionists target to increase Fe and Zn levels of wheat genotypes currently grown in the region by 25 and 10 mg/kg, respectively. On average, this translates into Zn and Fe levels in the grain of 35 and 50 mg/kg, respectively [10]. Bio-fortified high-Zn wheat could benefit 120 million resource poor people in South Asia, thus, providing sustainable solution to malnutrition problems by exploring natural genetic variation [11]. Therefore, improvement in the breeding techniques to increase the nutrient content of the staple cereal crops with micronutrient like Zn and Fe is a priority issue.

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In Nepal, 54 wheat varieties are released, among them only three biofortified (zinc and iron-enriched) wheat varieties (Zinc Gahun 1, Zinc Gahun 2 and Borlaug 2020) for Terai/Plains and three biofortified varieties (Himganga, Bheri-Ganga and Khumal-Shakti) for hills has been released till 2021 [12], [13]. This has provided base for the assumption that high percentage of Nepalese people are in the risk of Zn and Fe deficiency. Therefore, National Wheat Research Program, Bhairahawa in collaboration with International Maize and Wheat Improvement Center (CIMMYT) is testing and releasing zinc and iron enriched wheat varieties in Nepal. Biofortified wheat has several potential advantages as a delivery vehicle of zinc and iron through wheat in diets of Nepal where much of the wheat produced is milled locally and use of whole wheat meal in food products allows retaining most of the zinc and iron in the

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grain. The nutrient rich high yielding wheat cultivars offer the most economical and feasible means for improving micronutrient nutrition in rural areas. The main objective of this research was to evaluate advanced biofortified material and to identify high-yielding biofortified lines for terai region of Nepal.

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## 2. MATERIAL AND METHODS

### 2.1. Experimental design and procedure

During 2016/17 and 2017/18 wheat seasons 7th Harvest Plus Yield Trial (7HPYT) and 8th Harvest Plus Yield Trial (8HPYT), respectively were carried out at National Wheat Research Program, Bhairahawa. This research farm is geographically situated within latitude 27°31'49" N and longitude 83°27'36" E at altitude 105 m above sea level. The research was laid out in a  $\alpha$ -Lattice design with two replications. Each replication had 5 blocks i.e., 10 experimental units (plots). Each plot had 6 rows of 3-meter length. The planting material of 7HPYT and 8HPYT consisted of 50 genotypes, 47 of them were advanced lines obtained from CIMMYT with significantly improved Zn and Fe concentrations and desirable agronomic traits, two commercial checks [BAJ#1, KACHU#1] and one local check (Gautam). The genotypic detail is given in Annex 1 and 2. The genotypes which are used in this study were having *T. dicoccum* and *Aegilopes squarrosa* in their pedigree which are identified donors of high Fe and Zinc. The standard protocol recommended by National Wheat Research Program, Bhairahawa, Nepal was followed for all cultivation practices throughout the cropping season. Fertilizers application rate was 100:50:50 N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O kg ha<sup>-1</sup>. Data recording were carried on quantitative characteristics like days to heading, days to maturity, plant height, thousand grains weights, grain yield and biomass yield.

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### 2.2. Grain sampling and micronutrient determination

During harvesting, 20 spikes from each plot were hand plucked using gloves at physiological maturity. Afterwards, they were threshed carefully and were sorted for Zn and Fe analysis. Samples were monitored making sure to avoid metal contamination. About 20g grain samples were taken and examined to remove broken grains and foreign materials. After sorting, the samples were used for micronutrient analysis. Grain samples were analyzed using a non-destructive, bench-top, energy-dispersive X-ray fluorescence (EDXRF) machine (model x-supreme 800, Oxford Instruments plc, Abingdon, UK) standardized for high throughput screening of GZnC and GFeC (unit: mg kg<sup>-1</sup> or ppm) concentration in whole grain wheat (Paltridge et al., 2012). Micronutrient Analysis (Zn and Fe) was done at Banaras Hindu University (BHU), Vanarashi, India.

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### 2.3. Statistical analysis

Microsoft Office Excel 2007 was used for data entry and processing. Analysis of variance (ANOVA), correlation analysis, cluster analysis, principal component analysis (PCA), and calculation of means were conducted using R Studio software version 4.3.1. Statistical significance was determined at a 5% level of probability.

## 3. RESULTS AND DISCUSSIONS

### 3.1. 7thHPYT (Harvest Plus Yield Trial)

Days to heading, days to maturity, grain zinc and iron concentration, thousand grains weight and grain yield were highly significant ( $p \leq 0.01$ ) among the genotypes when compared with

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checks (Table 2). Additionally, plant height and biomass yield were also found significantly higher ( $p \leq 0.05$ ) among genotypes than the checks in 7th HPYT (Table 1).

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### 3.1.1. Days to heading, days to maturity and plant height

The days to heading among genotypes varied between 75 to 88 days with an average value of 83 days. The earliest heading was found in genotype 7HPYT404 followed by 7HPYT413, BAJ#1, 7HPYT416 and 7HPYT450. The Check Variety Gautam headed in 82 days. Similarly, the days to maturity varied among genotypes from 115 to 123 days with a mean value of 118 days. Genotype 7HPYT404, 7HPYT413, 7HPYT416, BAJ#1 and 7HPYT409 were found early maturing genotype with 115 days to maturity. The late maturing genotype was 7HPYT431 (123 days). The plant height varied from 78 to 91 cm. The shortest plant height was found in genotype 7HPYT409 (78 cm) followed by 7HPYT410, 7HPYT416 and 7HPYT420 with 80 cm. The check variety Gautam has plant height 88 cm (Table 1).

### 3.1.2. Grain zinc and iron concentration

Among the genotypes, the grain Zn concentration differed from 25.8 to 42.4 ppm with the mean of 32.07 ppm. The highest grain Zn concentration was found in genotype 7HPYT409 (42.4 ppm) followed by genotype 7HPYT410 (39.6 ppm). The lowest grain Zn concentration was found in 7HPYT412 (25.8 ppm). The grain Zn concentration of check variety Gautam was found 39.1 ppm (Table 1 and Figure 1).

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The grain Fe concentration was found between 20.6 to 57.9 ppm with a mean of 37.5 ppm. The highest grain Fe concentration was found in genotype 7HPYT442 (57.9 ppm) followed by 7HPYT410 (57.2 ppm), 7HPYT409 (54.7 ppm) and 7HPYT406 (49.6 ppm). The lowest grain Fe concentration was found in 7HPYT421 (20.6 ppm). The grain iron concentration of check variety Gautam was found 40.5 ppm (Table 1 and Figure 1).

### 3.1.3. Thousand grain weights, grain yield and biomass yield

The value of thousand grains weights ranged between 36 and 55 g with a mean of 43.9g. The highest thousand grains weight was found in genotype 7HPYT422 (55g) followed by genotypes 7HPYT440 (51g), 7HPYT415 (50g) while the lowest one was found in 7HPYT439 (36g). The check variety Gautam has thousand grain weight 46g. The grain yield varied among genotypes from 2205 - 3821 kg/ha with a mean value of 3100 kg/ha (Table 1). Highest grain yield was found in genotype 7HPYT448 (3821 kg/ha) followed by 7HPYT418 (3760 kg/ha), 7HPYT426 (3688 kg/ha), 7HPYT413 (3592 kg/ha), 7HPYT421 (3487 kg/ha), 7HPYT414 (3446 kg/ha), 7HPYT419 (3430 kg/ha) and 7HPYT424 (3416 kg/ha) while the lowest grain yield was found in 7HPYT410 (2205 kg/ha). The check variety Gautam produced grain yield of 3403 kg/ha. Similarly, the biomass yield varied among genotypes from 5801 to 8510 kg/ha with a mean value of 7055 kg/ha (Table 1). Genotype 7HPYT421 (8510 kg/ha) had the highest biomass yield followed by 7HPYT448 (8197 kg/ha), 7HPYT426 (8176 kg/ha), 7HPYT422 (8055 kg/ha) and kachu#1 (7968 kg/ha) while the lowest biomass yield was of genotype 7HPYT407 (5801 kg/ha). The check variety Gautam produced biomass yield of 7142 kg/ha.

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In 7th HPYT, out of all 50 biofortified wheat genotypes, 8 genotypes (i.e., 16% of genotypes) yielded more than local check (Gautam). 17 genotypes were selected and promoted to NAL (Nepal Advance line) and CB (Crossing Block). The grain yield percentage of all genotypes over local check is given in Figure 3.

**Table 1; Mean value of 7th HPYT entries for days to heading, days to maturity, plant height, 1000-grain weight, grain Zn and Fe concentrations, grain yield and Biomass Yield.**

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| E. N | Genotypes | DTH<br>(days) | DTM<br>(days) | PH<br>(cm) | TGW<br>(g) | GZnC<br>(ppm) | GFeC<br>(ppm) | GY<br>(kg/ha) | BY<br>(kg/ha) |
|------|-----------|---------------|---------------|------------|------------|---------------|---------------|---------------|---------------|
| 1    | GAUTAM    | 82            | 120           | 88         | 46         | 39.1          | 40.5          | 3403          | 7142          |
| 2    | BAJ #1    | 78            | 115           | 81         | 38         | 30.1          | 33.0          | 3102          | 7232          |
| 3    | KACHU #1  | 83            | 119           | 83         | 40         | 31.6          | 34.1          | 3401          | 7968          |
| 4    | 7HPYT404  | 75            | 115           | 85         | 44         | 34.9          | 43.6          | 2689          | 6277          |
| 5    | 7HPYT405  | 81            | 116           | 88         | 44         | 32.4          | 39.5          | 3136          | 7267          |
| 6    | 7HPYT406  | 86            | 119           | 88         | 47         | 37.3          | 49.6          | 2935          | 6840          |
| 7    | 7HPYT407  | 80            | 118           | 85         | 49         | 34.6          | 46.8          | 2610          | 5801          |
| 8    | 7HPYT408  | 85            | 118           | 83         | 48         | 37.2          | 40.4          | 2589          | 5877          |
| 9    | 7HPYT409  | 80            | 115           | 78         | 42         | 42.4          | 54.7          | 2572          | 6028          |
| 10   | 7HPYT410  | 86            | 119           | 80         | 48         | 39.6          | 57.2          | 2205          | 5845          |
| 11   | 7HPYT411  | 86            | 119           | 82         | 45         | 32.4          | 40.1          | 3205          | 7843          |
| 12   | 7HPYT412  | 85            | 120           | 83         | 36         | 25.8          | 30.7          | 2994          | 7095          |
| 13   | 7HPYT413  | 77            | 115           | 85         | 44         | 27.0          | 27.5          | 3592          | 7404          |
| 14   | 7HPYT414  | 82            | 118           | 88         | 43         | 28.5          | 33.4          | 3446          | 7848          |
| 15   | 7HPYT415  | 84            | 118           | 87         | 50         | 31.4          | 38.5          | 2842          | 6988          |
| 16   | 7HPYT416  | 78            | 115           | 80         | 45         | 27.7          | 33.8          | 3230          | 6581          |
| 17   | 7HPYT417  | 84            | 119           | 83         | 43         | 32.2          | 34.4          | 3244          | 7212          |
| 18   | 7HPYT418  | 85            | 119           | 81         | 50         | 29.1          | 33.0          | 3760          | 7868          |
| 19   | 7HPYT419  | 81            | 117           | 83         | 49         | 33.3          | 35.3          | 3430          | 7030          |
| 20   | 7HPYT420  | 83            | 119           | 80         | 44         | 30.2          | 32.9          | 2743          | 6535          |
| 21   | 7HPYT421  | 80            | 118           | 87         | 44         | 28.6          | 20.6          | 3487          | 8510          |
| 22   | 7HPYT422  | 86            | 118           | 88         | 43         | 31.7          | 40.3          | 3267          | 8055          |
| 23   | 7HPYT423  | 81            | 117           | 86         | 49         | 28.9          | 34.4          | 3271          | 6742          |
| 24   | 7HPYT424  | 87            | 117           | 85         | 46         | 26.1          | 31.6          | 3416          | 7150          |
| 25   | 7HPYT425  | 85            | 120           | 83         | 43         | 28.7          | 29.0          | 2885          | 6257          |
| 26   | 7HPYT426  | 86            | 120           | 89         | 47         | 37.2          | 43.0          | 3688          | 8176          |
| 27   | 7HPYT427  | 83            | 118           | 84         | 50         | 32.5          | 46.2          | 3266          | 7325          |
| 28   | 7HPYT428  | 82            | 119           | 82         | 39         | 35.1          | 31.2          | 3085          | 6890          |
| 29   | 7HPYT429  | 86            | 119           | 89         | 41         | 35.9          | 33.0          | 3354          | 7877          |
| 30   | 7HPYT430  | 84            | 119           | 86         | 40         | 27.7          | 35.1          | 3051          | 6764          |
| 31   | 7HPYT431  | 88            | 123           | 84         | 36         | 27.1          | 36.8          | 2243          | 6139          |
| 32   | 7HPYT432  | 79            | 116           | 82         | 43         | 30.3          | 36.2          | 2872          | 6906          |
| 33   | 7HPYT433  | 81            | 118           | 87         | 45         | 30.8          | 33.6          | 3311          | 7188          |
| 34   | 7HPYT434  | 87            | 119           | 89         | 44         | 31.5          | 38.3          | 3244          | 7094          |
| 35   | 7HPYT435  | 83            | 119           | 88         | 42         | 30.7          | 35.3          | 3047          | 6724          |
| 36   | 7HPYT436  | 85            | 119           | 87         | 40         | 38.7          | 40.4          | 3081          | 6856          |
| 37   | 7HPYT437  | 84            | 119           | 87         | 39         | 34.0          | 39.9          | 2928          | 6554          |
| 38   | 7HPYT438  | 82            | 119           | 87         | 41         | 32.1          | 34.5          | 2894          | 6544          |
| 39   | 7HPYT439  | 87            | 116           | 86         | 36         | 31.4          | 31.2          | 3310          | 7735          |

|                  |          |       |        |       |       |       |       |        |        |
|------------------|----------|-------|--------|-------|-------|-------|-------|--------|--------|
| 40               | 7HPYT440 | 83    | 118    | 88    | 51    | 32.7  | 39.1  | 2797   | 7166   |
| 41               | 7HPYT441 | 83    | 118    | 91    | 47    | 28.9  | 32.1  | 3158   | 7217   |
| 42               | 7HPYT442 | 88    | 120    | 90    | 55    | 38.6  | 57.9  | 2536   | 7357   |
| 43               | 7HPYT443 | 87    | 119    | 88    | 41    | 28.7  | 38.5  | 2940   | 6899   |
| 44               | 7HPYT444 | 88    | 119    | 87    | 41    | 32.8  | 36.3  | 3007   | 6616   |
| 45               | 7HPYT445 | 87    | 118    | 87    | 42    | 28.8  | 35.4  | 3473   | 7492   |
| 46               | 7HPYT446 | 88    | 119    | 85    | 42    | 31.0  | 37.6  | 2987   | 6504   |
| 47               | 7HPYT447 | 88    | 119    | 85    | 39    | 30.9  | 35.6  | 3086   | 6824   |
| 48               | 7HPYT448 | 82    | 116    | 85    | 43    | 30.6  | 34.5  | 3821   | 8197   |
| 49               | 7HPYT449 | 83    | 119    | 88    | 46    | 32.1  | 41.7  | 3054   | 6947   |
| 50               | 7HPYT450 | 78    | 117    | 82    | 43    | 33.3  | 39.0  | 3337   | 7379   |
| Grand Mean       |          | 83.42 | 118.03 | 85.31 | 43.91 | 32.07 | 37.55 | 3100.5 | 7055   |
| CV (%)           |          | 2.47  | 1.11   | 3.39  | 3.7   | 9.42  | 16.77 | 10.73  | 9.1    |
| LSD value (0.05) |          | 4.66  | 4.76   | 5.03  | 5.23  | 5.4   | 11.87 | 1086.8 | 1814.9 |
| P value          |          | <0.01 | <0.01  | 0.015 | <0.01 | <0.01 | 0.005 | 0.007  | 0.023  |

Where, DTH=days to heading, DTM=days to maturity, PH=plant height, TGW=Thousand grain weight, GFeC(ppm)=Grain Iron concentration, GZnC(ppm)=Grain Zinc concentration, GY=Grain yield, BY=Biomass Yield, CV=Coefficient of variation and LSD=least significant difference.

### 3.2. 8th HPYT (Harvest Plus Yield Trial)

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High significant differences ( $p \leq 0.01$ ) were found among the genotypes for days to heading, days to maturity, plant height, grain iron concentration, thousand grain weight and grain yield whereas significant difference ( $p \leq 0.05$ ) was noticed among the genotypes for grain zinc concentration when compared with the checks (Table 2).

#### 3.2.1. Days to heading, days to maturity and plant height

The days to heading among genotypes varied between 77 to 93 days with an average of 88 days. The earliest heading was found in genotype 8HPYT404 followed by 8HPYT429, 8HPYT421 and BAJ#1. The check variety Gautam headed in 85 days. Similarly, the days to maturity varied among genotypes from 116 to 122 days with a mean value of 120 days. Genotype 8HPYT404 (116 days), 8HPYT429 and 8HPYT435 were found early maturing genotype with 117 days to maturity. The plant height varied from 74 to 95 cm. Genotype 8HPYT440 (74 cm) had the shortest plant height followed by 8HPYT404 and 8HPYT428. The check variety Gautam had plant height of 84 cm (Table 2).

#### 3.2.2. Grain zinc and iron concentration

Grain Zn concentration was found varied from 23.8 to 37.5 ppm among the genotypes with the mean of 29.2 ppm. Highest Zn concentration was found in genotype 8HPYT417 (37.5 ppm) followed by genotype 8HPYT404 (34.4 ppm), 8HPYT443 (33.5 ppm) and 8HPYT433 (32.7 ppm). The lowest grain Zn concentration was found in 8HPYT421 (23.8 ppm). The grain zinc concentration of check variety Gautam was found 30.7 ppm (Table 2 and Figure 2).

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Similarly, the value of grain iron concentration varied between 28.9 to 60.6 ppm with a mean value of 32.7 ppm. The grain Fe concentration was found highest in genotype 8HPYT417 (60.6 ppm) followed by 8HPYT443 (38.1 ppm) and 8HPYT435 (37.8 ppm). The lowest grain Fe concentration was found in 8HPYT449 (28.9 ppm). The grain Fe concentration of check variety Gautam was found 36.3 ppm (Table 2 and Figure 2).

### 3.2.3. Thousand grain weights, grain yield and biomass yield

In the study, the weight of thousand grains varied from 34 and 50 g with a mean of 39.2g. Genotype 8HPYT421 (49.5g) had the highest thousand grains weight followed by genotypes 8HPYT431 (49g), 8HPYT406 (48g) and 8HPYT424 (45g). The check variety Gautam had thousand grains weight of 40g. The grain yield varied among genotypes from 2245 to 4456 kg/ha with a mean value of 3283 kg/ha (Table 2). The grain yield was found highest in genotype 8HPYT415 (4456 kg/ha) followed by 8HPYT431 (4079 kg/ha), 8HPYT429 (3964 kg/ha), 8HPYT407 (3959 kg/ha), and 8HPYT405 (3848 kg/ha) while the lowest grain yield was found in 8HPYT417 (2245 kg/ha). The check variety (Gautam) produced grain yield of 2648 kg/ha. Similarly, the biomass yield varied among genotypes from 5661 to 9582 kg/ha with a mean value of 7475 kg/ha (Table 2). The biomass yield was found highest in 8HPYT415 (9582 kg/ha) followed by 8HPYT431 (9172 kg/ha), 8HPYT408 (9000 kg/ha), 8HPYT407 (8936 kg/ha), 8HPYT450 (8805 kg/ha) and Kachu#1 (8770 kg/ha) while the lowest biomass yield was found in 8HPYT417 (5661 kg/ha). The check variety (Gautam) produced biomass yield of 6516 kg/ha.

In 8th HPYT, out of all 50 biofortified wheat genotypes, 48 genotypes (i.e., 96% of genotypes) yielded more than local check (Gautam). 38 genotypes were selected and promoted to Nepal Advance line (NAL), Nepal Rainfed Nursery (NRN), Initial Evaluation Trial-Biofortified (IET-B) and IET-TTL (Initial Evaluation Trial -Terai, Tar and Lower valley). The grain yield percentage of all genotypes over local check is given in Figure 3.

**Table 2: Mean value of 8th HPYT entries for days to heading, days to maturity, plant height, 1000-grain weight, grain Zn and Fe concentrations, grain yield and Biomass Yield.**

| E. N | Genotypes | DTH<br>(days) | DTM<br>(days) | PH<br>(cm) | TGW<br>(g) | GZnC<br>(ppm) | GFeC<br>(ppm) | GY<br>(kg/ha) | BY<br>(kg/ha) |
|------|-----------|---------------|---------------|------------|------------|---------------|---------------|---------------|---------------|
| 1    | GAUTAM    | 85            | 118           | 83.5       | 40.3       | 30.7          | 36.3          | 2648          | 6516          |
| 2    | BAJ #1    | 84            | 117           | 80.0       | 34.3       | 25.6          | 31.0          | 3296          | 7355          |
| 3    | KACHU #1  | 87            | 119           | 86.5       | 36.8       | 24.2          | 29.5          | 3409          | 8770          |
| 4    | 8HPYT404  | 77            | 116           | 76.0       | 38.0       | 34.4          | 35.9          | 2937          | 7000          |
| 5    | 8HPYT405  | 87            | 119           | 84.0       | 37.0       | 27.6          | 31.4          | 3848          | 7683          |
| 6    | 8HPYT406  | 86            | 119           | 83.5       | 47.8       | 32.0          | 34.5          | 2713          | 6755          |
| 7    | 8HPYT407  | 91            | 121           | 85.5       | 39.0       | 30.5          | 33.0          | 3959          | 8936          |
| 8    | 8HPYT408  | 90            | 121           | 93.5       | 40.5       | 28.2          | 32.9          | 3837          | 9000          |
| 9    | 8HPYT409  | 89            | 119           | 90.5       | 39.5       | 29.9          | 29.3          | 3328          | 7711          |
| 10   | 8HPYT410  | 91            | 121           | 88.0       | 37.8       | 28.4          | 30.7          | 2828          | 6378          |
| 11   | 8HPYT411  | 90            | 120           | 81.0       | 35.5       | 27.6          | 30.5          | 3168          | 6812          |
| 12   | 8HPYT412  | 88            | 120           | 85.0       | 35.8       | 29.4          | 31.5          | 3295          | 7328          |
| 13   | 8HPYT413  | 87            | 119           | 85.0       | 42.8       | 28.7          | 33.5          | 3156          | 6915          |
| 14   | 8HPYT414  | 89            | 119           | 82.5       | 42.3       | 29.5          | 32.4          | 3192          | 7190          |
| 15   | 8HPYT415  | 89            | 120           | 84.0       | 40.0       | 27.1          | 31.6          | 4456          | 9582          |
| 16   | 8HPYT416  | 87            | 120           | 89.5       | 41.3       | 26.7          | 30.4          | 3492          | 7857          |
| 17   | 8HPYT417  | 92            | 122           | 86.0       | 34.3       | 37.5          | 60.6          | 2245          | 5661          |
| 18   | 8HPYT418  | 89            | 121           | 89.5       | 40.3       | 27.7          | 31.8          | 3161          | 6833          |
| 19   | 8HPYT419  | 87            | 119           | 94.5       | 41.8       | 26.3          | 30.8          | 3835          | 7979          |
| 20   | 8HPYT420  | 88            | 119           | 85.5       | 37.0       | 29.3          | 33.0          | 3394          | 7000          |
| 21   | 8HPYT421  | 84            | 117           | 87.0       | 49.5       | 23.8          | 29.1          | 3831          | 8695          |

|                  |          |       |       |       |       |      |       |        |         |
|------------------|----------|-------|-------|-------|-------|------|-------|--------|---------|
| 22               | 8HPYT422 | 86    | 119   | 84.5  | 39.3  | 28.9 | 29.9  | 3000   | 6839    |
| 23               | 8HPYT423 | 88    | 119   | 85.0  | 42.3  | 30.6 | 30.1  | 3574   | 7817    |
| 24               | 8HPYT424 | 89    | 120   | 85.0  | 44.8  | 27.9 | 32.9  | 3078   | 6587    |
| 25               | 8HPYT425 | 93    | 122   | 82.0  | 44.5  | 32.1 | 33.3  | 2667   | 5833    |
| 26               | 8HPYT426 | 91    | 121   | 86.0  | 38.3  | 29.4 | 33.2  | 3007   | 6984    |
| 27               | 8HPYT427 | 93    | 121   | 88.0  | 40.5  | 31.3 | 34.0  | 3578   | 8254    |
| 28               | 8HPYT428 | 88    | 120   | 79.0  | 38.5  | 29.7 | 34.0  | 3084   | 6667    |
| 29               | 8HPYT429 | 83    | 117   | 88.0  | 44.5  | 25.8 | 33.3  | 3964   | 8529    |
| 30               | 8HPYT430 | 86    | 118   | 86.0  | 36.3  | 27.4 | 31.9  | 3333   | 7505    |
| 31               | 8HPYT431 | 90    | 120   | 88.0  | 48.8  | 29.4 | 34.9  | 4079   | 9172    |
| 32               | 8HPYT432 | 88    | 120   | 84.0  | 35.8  | 28.8 | 34.1  | 2908   | 7569    |
| 33               | 8HPYT433 | 87    | 120   | 83.5  | 37.8  | 32.7 | 33.6  | 3085   | 7418    |
| 34               | 8HPYT434 | 88    | 120   | 81.0  | 38.8  | 28.2 | 32.7  | 3667   | 8090    |
| 35               | 8HPYT435 | 84    | 117   | 82.5  | 42.5  | 31.3 | 37.8  | 3832   | 8447    |
| 36               | 8HPYT436 | 88    | 119   | 83.5  | 38.8  | 31.7 | 30.1  | 3075   | 6736    |
| 37               | 8HPYT437 | 91    | 120   | 82.5  | 38.5  | 27.2 | 29.9  | 2969   | 6918    |
| 38               | 8HPYT438 | 91    | 120   | 84.5  | 37.5  | 27.8 | 30.9  | 2667   | 5924    |
| 39               | 8HPYT439 | 88    | 119   | 92.0  | 34.3  | 27.6 | 32.1  | 3163   | 7839    |
| 40               | 8HPYT440 | 91    | 120   | 73.5  | 35.8  | 28.6 | 30.4  | 3498   | 8447    |
| 41               | 8HPYT441 | 91    | 121   | 84.0  | 36.3  | 28.6 | 31.3  | 3255   | 7661    |
| 42               | 8HPYT442 | 90    | 119   | 85.5  | 36.5  | 30.9 | 32.5  | 3003   | 6553    |
| 43               | 8HPYT443 | 89    | 119   | 89.5  | 42.3  | 33.5 | 38.1  | 2932   | 6284    |
| 44               | 8HPYT444 | 89    | 120   | 92.0  | 33.3  | 28.0 | 30.3  | 2765   | 6784    |
| 45               | 8HPYT445 | 91    | 120   | 88.0  | 34.8  | 29.4 | 32.6  | 3005   | 7328    |
| 46               | 8HPYT446 | 92    | 122   | 92.0  | 36.8  | 30.1 | 30.2  | 3474   | 8138    |
| 47               | 8HPYT447 | 92    | 121   | 87.0  | 36.0  | 27.8 | 30.1  | 3670   | 8720    |
| 48               | 8HPYT448 | 88    | 121   | 81.5  | 41.0  | 27.8 | 31.3  | 3075   | 6800    |
| 49               | 8HPYT449 | 90    | 122   | 91.5  | 40.3  | 29.2 | 28.9  | 3009   | 7133    |
| 50               | 8HPYT450 | 88    | 120   | 80.5  | 36.8  | 31.2 | 33.0  | 3708   | 8805    |
| Grand Mean       |          | 88.4  | 119.6 | 85.4  | 39.2  | 29.2 | 32.7  | 3283.0 | 7474.6  |
| CV (%)           |          | 1.58  | 0.93  | 4.64  | 4.2   | 8.86 | 8.88  | 8.68   | 9.48    |
| LSD value (0.05) |          | 4.13  | 2.13  | 8.49  | 3.43  | 6.33 | 4.77  | 780.72 | 2296.11 |
| P value          |          | <0.01 | <0.01 | 0.004 | <0.01 | 0.03 | <0.01 | <0.01  | <0.01   |

Where, DTH=days to heading, DTM=days to maturity, PH=plant height, TGW=Thousand grain weight, GFeC(ppm)=Grain Iron concentration, GZnC(ppm)=Grain Zinc concentration, GY=Grain yield, BY=Biomass Yield, CV=Coefficient of variation and LSD=least significant difference.

Micronutrient deficiency is alarming situation in Nepal. One of the reasons for growing Zn and Fe deficiencies in developing countries like Nepal is because of the food habit. Monotonous consumption of cereal-based foods which are low in micronutrient and have reduced bioavailability of Zn and Fe adds to the micronutrient deficiency in South Asia [7]. In this study, an approach to evaluate different biofortified wheat lines for grain Zn and Fe concentration, grain yield and associated component traits have been carried out.

The study has showed that the concentration of grain Zn varied from 25.8 to 42.4 ppm in 7th HPYT and 23.8 to 37.5 ppm in 8th HPYT among genotypes. The range of 29-39.5 ppm and 17-61 ppm was also reported by Velu et al.[10] and Velu et al.[9]. Also, the concentration of grain Fe ranged from 20.6 to 57.9 ppm in 7th HPYT and 28.9 to 60.6 ppm in 8th HPYT among genotypes (Figure 1 and 2). Similar results were reported in previous studies by Velu et al.[10] and Pant et al.[14]

Correlation between grain yield and Zn concentration was observed negative ( $r = -0.32^*$  in 7th HPYT and  $-0.43^{**}$  in 8th HPYT), which was also reported by Gomez-Becerra et al. (2010)[15] and Velu et al. (2012)[10]. The correlation between grain Fe with grain yield was significantly negative ( $-0.54^{**}$  in 7th HPYT and  $-0.33^*$  in 8th HPYT) similar to the results obtained by various authors in their studies [10], [15]. The correlation of plant height with grain yield was observed non-significant positive whereas it was non-significant negative with grain Zn and grain Fe concentration (Figure 4 and 5). This result was consistent with the study conducted by Srinivasa et al. (2014)[16]. The result illustrates that intake of Zn and Fe is higher when the plant height is lower as compared those plants with greater heights.

Genotypes expressed significantly different for days to maturity with the range of 115-123 days in 7th HPYT and 116-122 days in 8th HPYT. The results are similar to the findings of Pandey et al. (2021)[17] & Nainabasti et al. (2024)[18]. The correlation between days to maturity and grain yield was found to be negative but non-significant ( $r = -0.24$  in 7th HPYT and  $r = -0.17$  in 8th HPYT) (Figure 4 and 5). This finding is in contrast to finding reported by Asif et al. (2004)[19]. Thousand grain weights showed positive correlation with zinc and iron concentration which comply with the findings McDonald et al (2008)[20] and Pfeiffer & McClafferty (2007) [11]. They reported zinc and iron-enriched genotypes had higher thousand grains weights.

The highly significant positive correlation was found between grain zinc and iron concentration ( $r = 0.74^{**}$  in 7th HPYT and  $r = 0.67^{**}$  in 8th HPYT) (Figure 4 and 5). The observed result of high positive significant relation between grain Zn and grain Fe suggests that it is feasible to simultaneously improve both micronutrients. Similar result also reported by Velu et al. (2012)[10] and Pant et al. (2020) [21].

Grain yield of biofortified entries ranged between 2205 to 3821 kg/ha in 7th HPYT and 2245 to 4456 kg/ha in 8th HPYT with mean value higher than national average (3088 kg/ha) of Nepal. This shows that the biofortified genotypes are capable of producing higher grain yield with added micronutrient supplements in them as compared to the check 'Gautam' variety. Thus, the study prospects the enhancement of Zn and Fe content of wheat without really compromising the grain yield. Similar results were also demonstrated earlier by Velu et al. (2012) [10] and McDonald et al. (2008) [20].

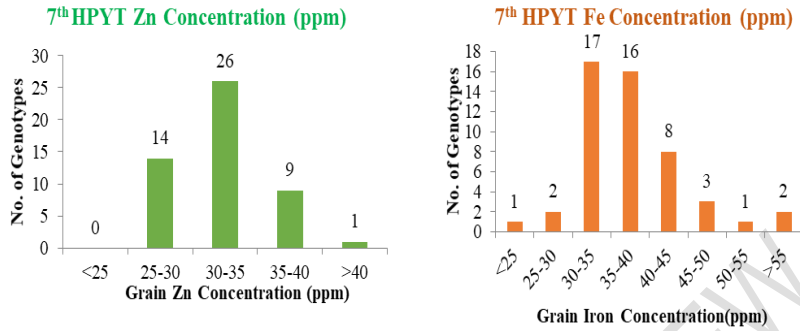


Figure 1: Frequency distribution for grain Zn and Fe concentrations of 7th HPYT entries

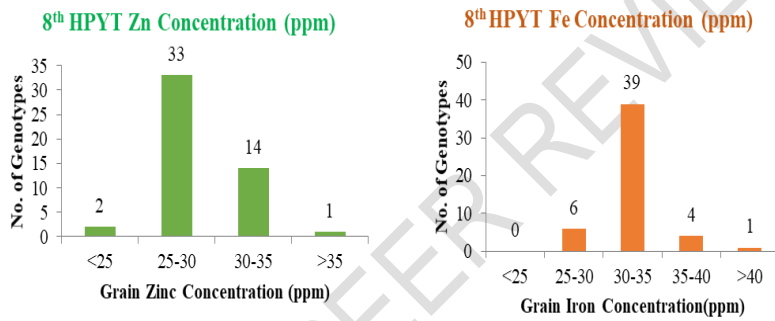


Figure 2: Frequency distribution for grain Zn and Fe concentrations of 8th HPYT entries

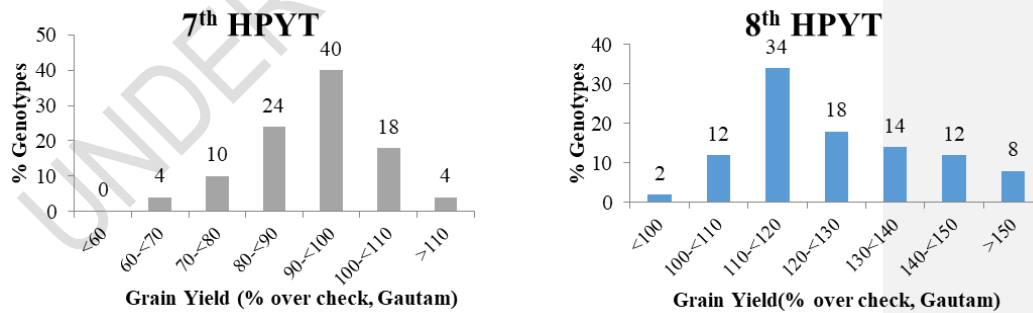
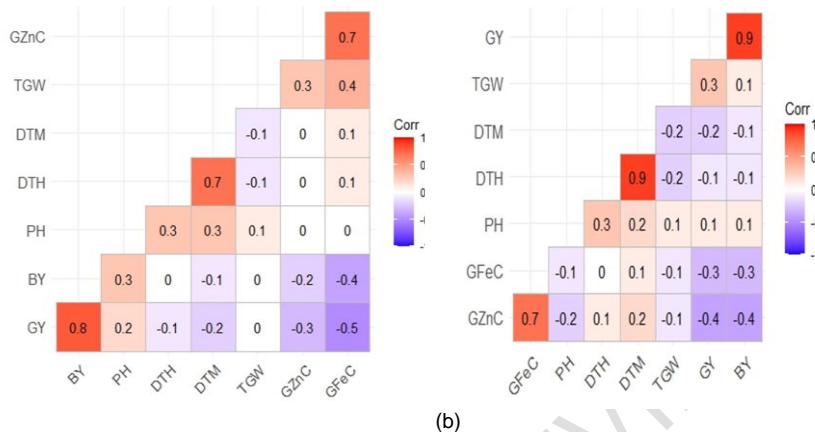


Figure 3: Increased grain yield of 7th HPYT and 8th HPYT entries over local check (Gautam)

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**Figure 4: (a) Distribution and correlation of morphological and yield related traits of 7th HPYT. (b) Distribution and correlation of morphological and yield related traits of 8th HPYT.**

Where, DTH=Days to heading, DTM=Days to maturity, PH=plant height, TGW=Thousand grain weight, GFeC(ppm)=Grain iron concentration, GZnC (ppm)=Grain Zinc concentration, GY=Grain yield and BY=Biomass yield, \*=Significant different at 5 % level of significance, \*\*=Highly Significant different at 1 % level of significance and \*\*\*=Highly Significant different at 0.1 % level of significance.

#### Principal Component Analysis (PCA):

##### In 7HPYT:

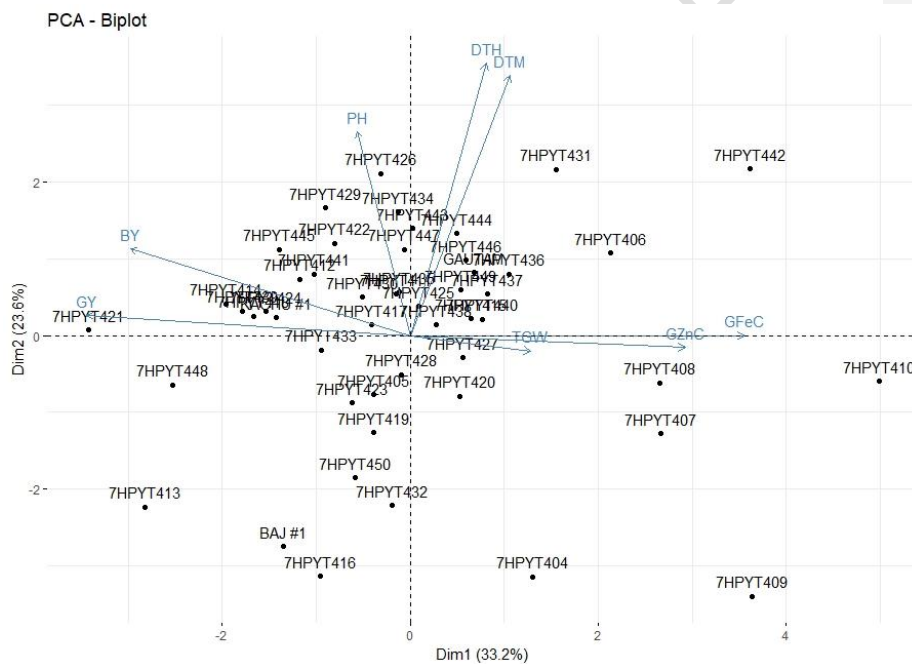
The PCA analysis indicated that the first five components (PC1, PC2, PC3, PC4 and PC5) explained maximum cumulative variances of 91.6% are important (Table 3). Among all PCs, the first PC (0.332) contributed maximum to the total variance. The major traits contributing to the first PC are grain zinc concentration, grain iron concentration, grain yield and biomass yield. Similarly, for second PC, DTH, DTM and PH were the major contributors. TGW and grain zinc concentration were the diversity contributor traits in the third PC. In fourth PC, max variation was explained by TGW followed grain zinc concentration. The PH and TGW are the major contributing traits for PC5.

The biplot explains the relationship of 50 wheat genotypes with component traits (Fig. 6). Across the 50 genotypes, grain yield was positively associated with biomass yield and negatively with grain zinc and iron concentration.

**Table 3: Vector loadings and proportion of variance explained by the first five principal components (PC) of 7th HPYT**

| Traits                 | PC1      | PC2      | PC3      | PC4      | PC5      |
|------------------------|----------|----------|----------|----------|----------|
| Days to heading        | 0.119883 | 0.622187 | 0.136391 | -0.14067 | -0.28514 |
| Days to maturity       | 0.156621 | 0.593864 | 0.223536 | 0.036227 | -0.24276 |
| Plant height           | -0.08343 | 0.465979 | -0.33484 | 0.318972 | 0.747138 |
| Thousand grains weight | 0.18922  | -0.03556 | -0.5929  | 0.584139 | -0.46786 |

|                          |          |          |          |          |          |
|--------------------------|----------|----------|----------|----------|----------|
| Grain Zinc Concentration | 0.432996 | -0.02719 | -0.34696 | -0.5752  | 0.163575 |
| Grain Iron Concentration | 0.526352 | 0.000257 | -0.29616 | -0.18181 | -0.01291 |
| Grain Yield              | -0.50924 | 0.045742 | -0.32785 | -0.24328 | -0.17798 |
| Biomass Yield            | -0.44099 | 0.197425 | -0.39014 | -0.33592 | -0.15511 |
| Loadings                 |          |          |          |          |          |
| Standard deviation       | 1.63     | 1.3738   | 1.2221   | 0.83578  | 0.77167  |
| Eigen value              | 2.65     | 1.887    | 1.493    | 0.697    | 0.594    |
| Proportion of Variance   | 0.332    | 0.2359   | 0.1867   | 0.08732  | 0.07443  |
| Cumulative Proportion    | 0.332    | 0.5679   | 0.7546   | 0.84188  | 0.91632  |



**Figure 56:** Principal Component analysis (PCA) between first and second principal components

#### 8HPYT:

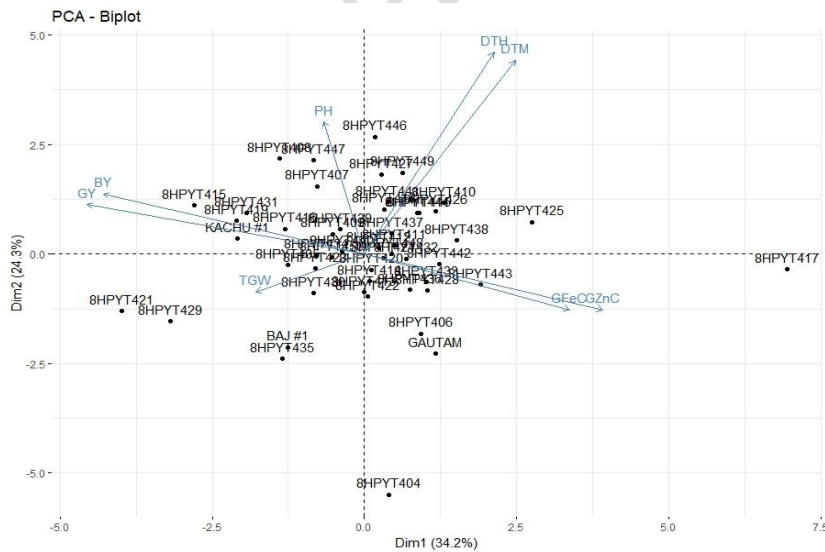
The PCA analysis indicated that the first five components (PC1, PC2, PC3, PC4 and PC5) explained maximum cumulative variances of 93.5% are important (Table 4). Among all PCs, the first PC (0.341) contributed maximum to the total variance. The major traits contributing to the first PC are grain zinc concentration, grain iron concentration, grain yield and biomass yield. Similarly, for second PC, DTH, DTM and PH were the major contributors. TGW, grain zinc concentration, grain iron concentration and grain yield were the diversity contributor traits

in the third PC. In fourth PC, max variation was explained by TGW followed by PH and biomass yield. The PH, TGW and grain iron concentration are the major contributing traits for PC5.

The biplot explains the relationship of 50 wheat genotypes with component traits (Fig. 7). Across the 50 genotypes, grain yield was positively associated with biomass yield and TGW and negatively with grain zinc and iron concentration.

**Table 4: Vector loadings and proportion of variance explained by the first five principal components (PC) of 8th HPYT**

| Traits                   | PC1      | PC2      | PC3      | PC4      | PC5      |
|--------------------------|----------|----------|----------|----------|----------|
| Days to heading          | 0.237536 | 0.609792 | -0.02221 | -0.08913 | -0.25302 |
| Days to maturity         | 0.276181 | 0.58614  | -0.06526 | -0.08583 | -0.24613 |
| Plant height             | -0.07398 | 0.3997   | -0.18596 | 0.568488 | 0.66698  |
| Thousand grains weight   | -0.19959 | -0.11401 | -0.53465 | 0.571162 | -0.56526 |
| Grain Zinc Concentration | 0.437355 | -0.16797 | -0.44355 | -0.19487 | -0.00615 |
| Grain Iron Concentration | 0.376994 | -0.1684  | -0.54114 | -0.18173 | 0.315146 |
| Grain Yield              | -0.50922 | 0.149227 | -0.31055 | -0.31304 | -0.00037 |
| Biomass Yield            | -0.47883 | 0.181638 | -0.29799 | -0.40779 | 0.107987 |
| Loadings                 |          |          |          |          |          |
| Standard deviation       | 1.6535   | 1.3951   | 1.066    | 0.974    | 0.84643  |
| Eigen value              |          |          |          |          |          |
| Proportion of Variance   | 0.3417   | 0.2433   | 0.142    | 0.1186   | 0.08956  |
| Cumulative Proportion    | 0.3417   | 0.585    | 0.727    | 0.8456   | 0.93518  |



**Figure 67: Principal Component analysis (PCA) between first and second principal components**

## Cluster Analysis

Cluster analysis helps plant breeders in identifying genetically diverse parents who fall into different clusters. Cluster analysis or clustering is the process of grouping, categorizing or classifying a set of objects into many subsets called clusters in such a way that items within one subset are more “similar” to each other, while items within other subsets are “dissimilar.” In 7th HPYT, genotypes were clustered based on variables: days to heading, days to maturity, plant height, thousand grains weight, grain zinc concentration, grain iron concentration, grain yield and biomass yield by Hierarchical clustering method. The mean values of clusters are presented in Table 5. The dendrogram constructed using R-studio software version 4.3.1 revealed four major clusters (Fig. 2).

Cluster I consisted of 13 genotypes, which represents 26% of total genotypes. It includes 7HPYT403, 7HPYT413, 7HPYT417, 7HPYT419, 7HPYT424, 7HPYT427, 7HPYT433, 7HPYT434, 7HPYT441, 7HPYT445, 7HPYT450, Baj#1 and Gautam. This cluster represents genotypes with highest TGW (44.7 g) and earliest in heading and maturity. The genotypes under this cluster are moderate in grain zinc and iron concentration and second in grain yield and biomass yield. Similarly, Cluster II consisted of 10 genotypes, which represents 20% of total genotypes. It includes 7HPYT411, 7HPYT414, 7HPYT418, 7HPYT421, 7HPYT422, 7HPYT426, 7HPYT429, 7HPYT439, 7HPYT448 and Kachu#1. This cluster represents genotypes with highest in grain and biomass yield.

Cluster III consisted of 22 genotypes, which represents 44% of total genotypes. It includes 7HPYT404, 7HPYT406, 7HPYT412, 7HPYT415, 7HPYT416, 7HPYT420, 7HPYT423, 7HPYT425, 7HPYT428, 7HPYT430, 7HPYT432, 7HPYT435, 7HPYT436, 7HPYT437, 7HPYT438, 7HPYT440, 7HPYT442, 7HPYT443, 7HPYT444, 7HPYT446, 7HPYT447 and 7HPYT449. This cluster represents genotypes with average value for all traits under study.

Cluster IV consisted of 5 genotypes, which represents 10 % of total genotypes. It includes 7HPYT407, 7HPYT408, 7HPYT409, 7HPYT410 and 7HPYT431. This cluster is characterized with genotypes having lowest grain and biomass yield and highest grain zinc and iron concentration. The mean value of each trait for each cluster was presented in Table 5.

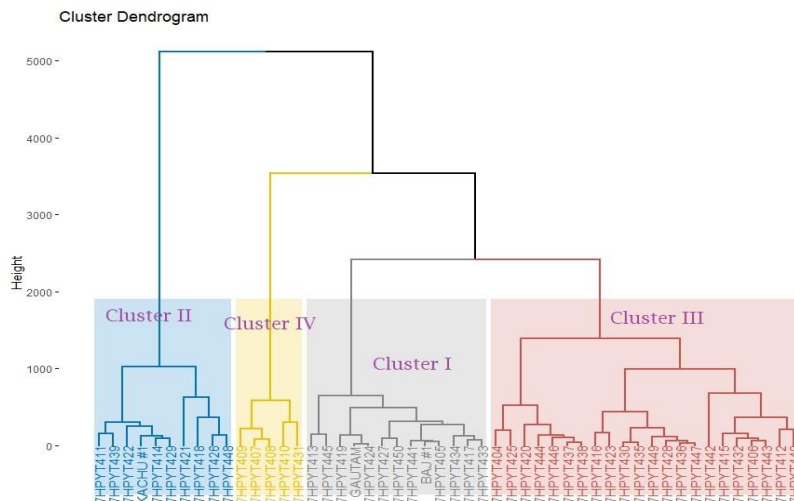
**Commented [mm24]:** From the four clusters, which have you qualified the most interesting in this study? And Why?

**Commented [mm25]:** To compare your finding with those of other works

**Commented [mm26]:** Make hypothesis or the finding of others work to explain your results

**Table 5: Mean value of traits for 4 clusters obtained from Hierarchical cluster analysis (7th HPYT)**

| Traits | Cluster I | Cluster II | Cluster III | Cluster IV | Grand Centroid |
|--------|-----------|------------|-------------|------------|----------------|
| DTH    | 82        | 84         | 84          | 84         | 84             |
| DTM    | 118       | 118        | 119         | 119        | 118            |
| PH     | 85.6      | 85.8       | 85.5        | 82.0       | 84.7           |
| TGW    | 44.7      | 43.2       | 43.5        | 44.6       | 44.0           |
| GZnC   | 31.2      | 31.7       | 31.8        | 36.2       | 32.7           |
| GFeC   | 35.9      | 34.3       | 37.8        | 47.2       | 38.8           |
| GY     | 3316.3    | 3473.9     | 2952.5      | 2443.8     | 3046.6         |
| BY     | 7240.9    | 8007.7     | 6766.6      | 5938.0     | 6988.3         |



**Figure 78: Clustering of genotypes based on Hierarchical clustering method (7th HPYT)**

In 8th HPYT, genotypes were clustered based on variables: days to heading, days to maturity, plant height, thousand grains weight, grain zinc concentration, grain iron concentration, grain yield and biomass yield by Hierarchical clustering method. The mean values of clusters are presented in Table 5.

Cluster I consisted of 20 genotypes, which represents 40% of total genotypes. It includes 8HPYT404, 8HPYT406, 8HPYT410, 8HPYT411, 8HPYT413, 8HPYT417, 8HPYT418, 8HPYT422, 8HPYT424, 8HPYT425, 8HPYT426, 8HPYT428, 8HPYT436, 8HPYT437, 8HPYT438, 8HPYT442, 8HPYT443, 8HPYT444, 8HPYT448 and Gautam. This cluster represents genotypes with highest grain zinc and iron concentration and lowest grain yield and biomass yield. Similarly, Cluster II consisted of 12 genotypes, which represents 24% of total genotypes. It includes 8HPYT409, 8HPYT412, 8HPYT414, 8HPYT420, 8HPYT430, 8HPYT432, 8HPYT433, 8HPYT439, 8HPYT441, 8HPYT445, 8HPYT449 and Baj#1. This cluster represents genotypes with average value for all traits under study.

Cluster III consisted of 10 genotypes, which represents 20% of total genotypes. It includes 8HPYT407, 8HPYT408, 8HPYT415, 8HPYT421, 8HPYT429, 8HPYT431, 8HPYT435, 8HPYT447 and 8HPYT450. This cluster represents genotypes with highest in TGW, grain yield and biomass yield while lowest in grain zinc concentration.

Cluster IV consisted of 8 genotypes, which represents 16 % of total genotypes. It includes 8HPYT405, 8HPYT416, 8HPYT419, 8HPYT423, 8HPYT427, 8HPYT434, 8HPYT440 and 8HPYT446. This cluster is characterized with genotypes second in grain yield and biomass yield and moderate in grain zinc and iron concentration. The mean value of each trait for each cluster was presented in Table 6.

**Table 6: Mean value of traits for 4 clusters obtained from Hierarchical cluster analysis (8th HPYT)**

| Traits | Cluster I | Cluster II | Cluster III | Cluster IV | Grand Centroid |
|--------|-----------|------------|-------------|------------|----------------|
|--------|-----------|------------|-------------|------------|----------------|

**Commented [mm27]:** Which are the relationship between the fertilizers application and the Zn and Fe concentrations in grains and other parameters?

**Commented [mm28]:** Based on the results of this study, is it interesting to have high grain yield with low Zn and Fe concentration or inversely? Specify your objective.

|      |        |        |        |        |        |
|------|--------|--------|--------|--------|--------|
| DTH  | 89     | 88     | 88     | 89     | 88     |
| DTM  | 120    | 120    | 119    | 120    | 120    |
| PH   | 84.4   | 86.0   | 86.3   | 85.9   | 85.7   |
| TGW  | 39.5   | 37.0   | 41.4   | 39.3   | 39.3   |
| GZnC | 30.1   | 29.0   | 27.9   | 28.7   | 28.9   |
| GFeC | 34.0   | 31.8   | 32.5   | 31.3   | 32.4   |
| GY   | 2908.9 | 3188.6 | 3874.5 | 3620.8 | 3398.2 |
| BY   | 6589.0 | 7419.8 | 8865.6 | 8033.1 | 7726.9 |

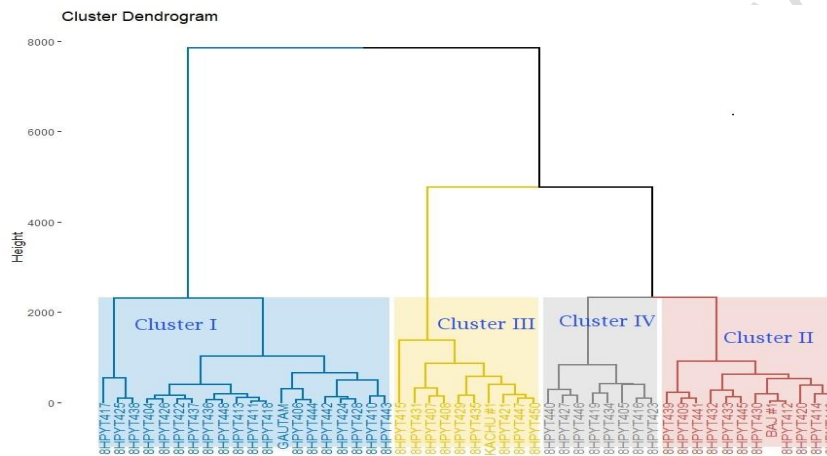


Figure 89: Clustering of genotypes based on Hierarchical clustering method (8th HPYT)

#### 4. Conclusion

This research showed the existence of large variability for grain zinc and iron concentration and grain yield among the tested biofortified genotypes. The appreciable number of entries exceeded the intermediate to full target level of grain Zn and Fe and grain yield in both 7th HPYT and 8th HPYT trials. Thus, competitive biofortified wheat varieties can be developed with competitive yields and other farmer-preferred agronomic traits. The genotypes with higher grain Zn and Fe concentration viz., 7HPYT409, 7HPYT410, 8HPYT417, 8HPYT404 and 7HPYT442 could be used as donor parents in national wheat breeding program and high yielding genotypes 7HPYT448, 7HPYT418, 7HPYT426, 7HPYT413, 8HPYT415, 8HPYT431, 8HPYT429, 8HPYT407 and 8HPYT405 would be further evaluated throughout the terai region of Nepal, and outstanding genotype could be released as variety for terai/plains of Nepal.

Commented [mm29]: Please, Change this word by fertilization or others

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#### REFERENCES

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**Annex 1: Genotypic details of 7HPYT (2016/17)**

| E.N. | Name of entries      | Cross Name   | Selection History                                   | Origin                |
|------|----------------------|--|---|-----------------------|
| 1    | GAUTAM (Local Check) | SIDDHARTH/NING8319/NL297   | -   | NEPAL                 |
| 2    | BAJ #1               | BAJ #1   | CGSS01Y00134<br>S-099Y-099M-099M-13Y-0B             | MXI15-16\M7THHPY T\32 |
| 3    | KACHU #1             | KACHU #1   | CMSS97M0391<br>2T-040Y-020Y-030M-020Y-040M-4Y-2M-0Y | MXI15-16\M7THHPY T\31 |
| 4    | 7HPYT404             | CROC_1/AE.SQUARROSA (210)//INQALAB 91*2/KUKUNA/3/PBW343*2/KUKUNA   | CMSA06M0019<br>5T-099Y-099Y-9M-0Y-7B-0Y             | MXI15-16\M7THHPY T\1  |
| 5    | 7HPYT405             | T.DICOCCON CI9309/AE.SQUARROSA (409)//MUTUS/3/2*MUTUS  | CMSS08Y01129<br>T-099M-099Y-3M-0Y-5M-0Y             | MXI15-16\M7THHPY T\2  |
| 6    | 7HPYT406             | DANPHE #1*2/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//SHA4/CHIL/4/WBLL1*2/KURUKU//HEILO/5/WBLL1*2/KURUKU//HEILO | CMSS11B01191<br>T-099TOPY-099M-099Y-3M-0WGY         | MXI15-16\M7THHPY T\5  |
| 7    | 7HPYT407             | DANPHE #1*2/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//SHA4/CHIL/4/WBLL1*2/KURUKU//HEILO/5/WBLL1*2/KURUKU//HEILO | CMSS11B01191<br>T-099TOPY-099M-099Y-30M-0WGY        | MXI15-16\M7THHPY T\7  |
| 8    | 7HPYT408             | DANPHE #1*2/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//SHA4/CHIL/4/WBLL1*2/KURUKU//HEILO/5/WBLL1*2/KURUKU//HEILO | CMSS11B01191<br>T-099TOPY-099M-099Y-31M-0WGY        | MXI15-16\M7THHPY T\8  |

|    |          |  |  |                       |
|----|----------|--|--|-----------------------|
| 9  | 7HPYT409 | CHONTE*2/SOLALA//2*BAJ #1  | CMSS11B01204<br>T-099TOPY-099M-099Y-8M-0WGY  | MXI15-16\M7THHPY T\24 |
| 10 | 7HPYT410 | FRNCLN*2/7/CMH83.1020/HUITES/6/CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/8/WBLL1*2/KURUKU//HEILO/9/WBLL1*2/KURUKU//HEILO                             | CMSS11B01210<br>T-099TOPY-099M-099Y-9M-0WGY  | MXI15-16\M7THHPY T\30 |
| 11 | 7HPYT411 | FRNCLN*2/7/CMH83.1020/HUITES/6/CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/8/WBLL1*2/KURUKU//HEILO/9/WBLL1*2/KURUKU//HEILO                             | CMSS11B01210<br>T-099TOPY-099M-099Y-16M-0WGY | MXI15-16\M7THHPY T\33 |
| 12 | 7HPYT412 | FRNCLN*2/7/CMH83.1020/HUITES/6/CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/8/WBLL1*2/KURUKU//HEILO/9/WBLL1*2/KURUKU//HEILO                             | CMSS11B01210<br>T-099TOPY-099M-099Y-51M-0WGY | MXI15-16\M7THHPY T\39 |
| 13 | 7HPYT413 | FRNCLN*2/7/CMH83.1020/HUITES/6/CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/8/KIRITATI/4/2*BAV92//IRENA/KAUZ/3/HUITES/9/FRANCOLIN #1//WBLL1*2/BRAMBLING | CMSS11B01213<br>T-099TOPY-099M-099Y-8M-0WGY  | MXI15-16\M7THHPY T\41 |
| 14 | 7HPYT414 | VILLA JUAREZ F2009/SOLALA//WBLL1*2/BRAMBLING/5/WAXWING/3/BL1496/MILAN//PI610750/4/FRNCLN/6/MUNAL/3/HUW234+LR34/PRINIA//PFAU/WEAVER                       | CMSS11B01216<br>T-099TOPY-099M-099Y-19M-0WGY | MXI15-16\M7THHPY T\46 |
| 15 | 7HPYT415 | KVZ/PPR47.89C//TACUPETO F2001*2/BRAMBLING/3/2*TACUPETO F2001*2/BRAMBLING/4/KACHU/5/KACHU #1/3/C80.1/3*BATAVIA//2*WBLL1/4/KACHU                           | CMSS11B01218<br>T-099TOPY-099M-099Y-7M-0WGY  | MXI15-16\M7THHPY T\47 |
| 16 | 7HPYT416 | KVZ/PPR47.89C//FRANCOLIN #1/3/2*PAURAQ/4/PBW343*2/KUKUNA*2//FRTL/PIFED/5/MUNAL #1  | CMSS11B01222<br>T-099TOPY-099M-099Y-29M-0WGY | MXI15-16\M7THHPY T\50 |

|    |              |  |  |                              |
|----|--------------|--|--|------------------------------|
| 17 | 7HPYT41<br>7 | HGO94.7.1.12/2*QUAIU<br>#1//QUAIU #2/3/KINGBIRD<br>#1//INQALAB<br>91*2/TUKURU/4/SUP152/BAJ<br>#1   | CMSS11B01227<br>T-099TOPY-<br>099M-099Y-<br>13M-0WGY | MXI15-<br>16\M7THHPY<br>T\51 |
| 18 | 7HPYT41<br>8 | HGO94.7.1.12/2*QUAIU<br>#1//QUAIU<br>#2/5/KIRITATI/4/2*BAV92//IR<br>ENA/KAUZ/3/HUITES/6/MUC<br>UY  | CMSS11B01228<br>T-099TOPY-<br>099M-099Y-<br>14M-0WGY | MXI15-<br>16\M7THHPY<br>T\53 |
| 19 | 7HPYT41<br>9 | HGO94.7.1.12/2*QUAIU<br>#1//QUAIU<br>#2/5/KIRITATI/4/2*BAV92//IR<br>ENA/KAUZ/3/HUITES/6/MUC<br>UY  | CMSS11B01228<br>T-099TOPY-<br>099M-099Y-<br>20M-0WGY | MXI15-<br>16\M7THHPY<br>T\54 |
| 20 | 7HPYT42<br>0 | CHIH95.2.6//WBLL1*2/KURU<br>KU/3/WBLL1*2/KKTS/4/ND64<br>3/2*WBLL1/5/SAUAL/YANAC<br>//SAUAL/6/WBLL1*2/BRAMB<br>LING//VORB/FISCAL/3/BECA<br>RD         | CMSS11B01230<br>T-099TOPY-<br>099M-099Y-<br>19M-0WGY | MXI15-<br>16\M7THHPY<br>T\55 |
| 21 | 7HPYT42<br>1 | CHIH95.2.6//WBLL1*2/KURU<br>KU/3/WBLL1*2/KKTS/4/ND64<br>3/2*WBLL1/5/TACUPETO<br>F2001/BRAMBLING*2//KACH<br>U/6/KUTZ                                  | CMSS11B01231<br>T-099TOPY-<br>099M-099Y-<br>12M-0WGY | MXI15-<br>16\M7THHPY<br>T\56 |
| 22 | 7HPYT42<br>2 | T.DICOCCON<br>CI9309/AE.SQUARROSA<br>(409)//2*PANDORA/3/KINGBI<br>RD #1//INQALAB<br>91*2/TUKURU/5/MUNAL/3/KI<br>RITATI//PRL/2*PASTOR/4/MU<br>NAL     | CMSS11B01236<br>T-099TOPY-<br>099M-099Y-<br>31M-0WGY | MXI15-<br>16\M7THHPY<br>T\60 |
| 23 | 7HPYT42<br>3 | T.DICOCCON<br>CI9309/AE.SQUARROSA<br>(409)//2*PANDORA/5/WAXWI<br>NG/3/BL 1496/MILAN//PI<br>610750/4/FRNCLN/6/KACHU/<br>BECARD//WBLL1*2/BRAMB<br>LING | CMSS11B01237<br>T-099TOPY-<br>099M-099Y-3M-<br>0WGY  | MXI15-<br>16\M7THHPY<br>T\63 |
| 24 | 7HPYT42<br>4 | HGO94.7.1.12/2*QUAIU<br>#1//WAXBI/5/WBLL1*2/4/BAB<br>AX/LR42//BABAX/3/BABAX/L<br>R42//BABAX  | CMSS11B01246<br>T-099TOPY-<br>099M-099Y-<br>23M-0WGY | MXI15-<br>16\M7THHPY<br>T\74 |

|    |          |  |  |                        |
|----|----------|--|--|------------------------|
| 25 | 7HPYT425 | COAH90.26.31/4/2*BL2064//SW89-5124*2/FASAN/3/TILHI/5/UP2338*2/KKTS*2//YANAC/6/MUTUS/AKURI  | CMSS11B01249<br>T-099TOPY-099M-099Y-19M-0WGY | MXI15-16\M7THHPY T\80  |
| 26 | 7HPYT426 | COAH90.26.31/4/2*BL2064//SW89-5124*2/FASAN/3/TILHI/5/UP2338*2/KKTS*2//YANAC/6/MUTUS/AKURI  | CMSS11B01249<br>T-099TOPY-099M-099Y-32M-0WGY | MXI15-16\M7THHPY T\82  |
| 27 | 7HPYT427 | CROC_1/AE.SQUARROSA (210)//INQALAB 91*2/KUKUNA/3/PBW343*2/KUKUNA/5/SAUAL/3/C80.1/3*BATAVIA//2*WBLL1/4/SITE/MO//PASTOR/3/TILHI/6/SAUAL#1/KACHU  | CMSS11B01270<br>T-099TOPY-099M-099Y-11M-0WGY | MXI15-16\M7THHPY T\89  |
| 28 | 7HPYT428 | UC1113-GPCB1/3/TACUPETO F2001/BRAMBLING*2//KACHU/4/TACUPETO F2001/BRAMBLING//KACHU   | CMSS11B01295<br>T-099TOPY-099M-099Y-10M-0WGY | MXI15-16\M7THHPY T\101 |
| 29 | 7HPYT429 | CHONTE*2/SOLALA/5/GARZA/BOY//AE.SQUARROSA (467)/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR/4/T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR/6/ATTILA*2/PBW65//PIHA/3/ATTILA/2*PASTOR | CMSS11B01302<br>T-099TOPY-099M-099Y-8M-0WGY  | MXI15-16\M7THHPY T\102 |
| 30 | 7HPYT430 | QUAIU #1/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR/4/QUAIU #2/5/BORL14   | CMSS11B01045<br>S-099M-099Y-1M-0WGY          | MXI15-16\M7THHPY T\107 |
| 31 | 7HPYT431 | DANPHE #1*2/SOLALA/3/ATTILA*2/PBW65//MURGA   | CMSS11B01057<br>S-099M-099Y-17M-0WGY         | MXI15-16\M7THHPY T\109 |
| 32 | 7HPYT432 | VILLA JUAREZ F2009/SOLALA//WBLL1*2/BRAMBLING/4/COAH90.26.31//KIRITATI/WBLL1/3/KIRITATI/2*WBLL1   | CMSS11B01079<br>S-099M-099Y-16M-0WGY         | MXI15-16\M7THHPY T\115 |

|    |              |   |  |                               |
|----|--------------|---|--|-------------------------------|
| 33 | 7HPYT43<br>3 | VILLA JUAREZ<br>F2009/SOLALA//WBLL1*2/BR<br>AMBLING/3/PBW343*2/KUK<br>UNA*2//FRTL/PIFED   | CMSS11B01081<br>S-099M-099Y-<br>11M-0WGY | MXI15-<br>16\M7THHPY<br>T\123 |
| 34 | 7HPYT43<br>4 | VILLA JUAREZ<br>F2009/SOLALA//WBLL1*2/BR<br>AMBLING/3/PBW343*2/KUK<br>UNA*2//FRTL/PIFED   | CMSS11B01081<br>S-099M-099Y-<br>15M-0WGY | MXI15-<br>16\M7THHPY<br>T\124 |
| 35 | 7HPYT43<br>5 | T.DICOCCON<br>CI9309/AE.SQUARROSA<br>(409)//MUTUS/3/2*MUTUS/5/T<br>.DICOCCON<br>PI94624/AE.SQUARROSA<br>(409)//BCN/3/WAXWING/4/2*<br>FRNCLN                                       | CMSS11B01083<br>S-099M-099Y-<br>21M-0WGY | MXI15-<br>16\M7THHPY<br>T\128 |
| 36 | 7HPYT43<br>6 | T.DICOCCON<br>CI9309/AE.SQUARROSA<br>(409)//MUTUS/3/2*MUTUS/4/F<br>RET2/TUKURU//FRET2*2/3/T.<br>SPELTA PI348530   | CMSS11B01084<br>S-099M-099Y-<br>6M-0WGY  | MXI15-<br>16\M7THHPY<br>T\130 |
| 37 | 7HPYT43<br>7 | T.DICOCCON<br>CI9309/AE.SQUARROSA<br>(409)//MUTUS/3/2*MUTUS/4/F<br>RET2/TUKURU//FRET2*2/3/T.<br>SPELTA PI348530   | CMSS11B01084<br>S-099M-099Y-<br>42M-0WGY | MXI15-<br>16\M7THHPY<br>T\133 |
| 38 | 7HPYT43<br>8 | T.DICOCCON<br>CI9309/AE.SQUARROSA<br>(409)//MUTUS/3/2*MUTUS/5/P<br>FAU/WEAVER*2/4/BOW/NKT<br>//CBRD/3/CBRD  | CMSS11B01087<br>S-099M-099Y-<br>21M-0WGY | MXI15-<br>16\M7THHPY<br>T\136 |
| 39 | 7HPYT43<br>9 | T.DICOCCON<br>PI94624/AE.SQUARROSA<br>(409)//BCN/3/WAXWING/4/2*<br>FRNCLN/5/VILLA JUAREZ<br>F2009/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/WBLL1*2/<br>BRAMBLING | CMSS11B01090<br>S-099M-099Y-<br>1M-0WGY  | MXI15-<br>16\M7THHPY<br>T\139 |
| 40 | 7HPYT44<br>0 | CHIH95.2.6/4/BABAX/LR42//B<br>ABAX*2/3/SHAMA/5/2*BABA<br>X/LR42//BABAX*2/3/TUKUR<br>U/6/KFA/2*KACHU   | CMSS11B01099<br>S-099M-099Y-<br>5M-0WGY  | MXI15-<br>16\M7THHPY<br>T\141 |

|    |              |  |  |                               |
|----|--------------|--|--|-------------------------------|
| 41 | 7HPYT44<br>1 | HGO94.7.1.12/2*QUAIU<br>#1/3/VILLA JUAREZ<br>F2009/SOLALA//WBLL1*2/BR<br>AMBLING   | CMSS11B01126<br>S-099M-099Y-<br>8M-0WGY  | MXI15-<br>16\M7THHPY<br>T\149 |
| 42 | 7HPYT44<br>2 | HGO94.7.1.12//WBLL1*2/KUK<br>UNA/3/WBLL1*2/KURUKU/4/<br>PBW343*2/KUKUNA*2//FRTL<br>/PIFED  | CMSS11B01134<br>S-099M-099Y-<br>9M-0WGY  | MXI15-<br>16\M7THHPY<br>T\154 |
| 43 | 7HPYT44<br>3 | COAH90.26.31//KIRITATI/WB<br>LL1/3/KIRITATI/2*WBLL1/7/O<br>ASIS/SKAUZ//4*BCN/3/2*PAS<br>TOR/4/T.SPELTA<br>PI348449/5/BACEU<br>#1/6/WBLL1*2/CHAPIO                                      | CMSS11B01145<br>S-099M-099Y-<br>19M-0WGY | MXI15-<br>16\M7THHPY<br>T\158 |
| 44 | 7HPYT44<br>4 | VILLA JUAREZ<br>F2009/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/WBLL1*2/<br>BRAMBLING/5/QUAIU<br>#1/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/QUAIU #2 | CMSS11B01149<br>S-099M-099Y-<br>4M-0WGY  | MXI15-<br>16\M7THHPY<br>T\160 |
| 45 | 7HPYT44<br>5 | VILLA JUAREZ<br>F2009/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/WBLL1*2/<br>BRAMBLING/5/QUAIU<br>#1/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/QUAIU #2 | CMSS11B01149<br>S-099M-099Y-<br>15M-0WGY | MXI15-<br>16\M7THHPY<br>T\161 |
| 46 | 7HPYT44<br>6 | VILLA JUAREZ<br>F2009/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/WBLL1*2/<br>BRAMBLING/5/QUAIU<br>#1/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/QUAIU #2 | CMSS11B01149<br>S-099M-099Y-<br>33M-0WGY | MXI15-<br>16\M7THHPY<br>T\164 |
| 47 | 7HPYT44<br>7 | VILLA JUAREZ<br>F2009/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/WBLL1*2/<br>BRAMBLING/7/OASIS/SKAU<br>Z//4*BCN/3/2*PASTOR/4/T.SP                                       | CMSS11B01151<br>S-099M-099Y-<br>21M-0WGY | MXI15-<br>16\M7THHPY<br>T\165 |

|    |              |  |  |                               |
|----|--------------|--|--|-------------------------------|
|    |              | ELTA PI348449/5/BACEU<br>#1/6/WBLL1*2/CHAPIO   |  |                               |
| 48 | 7HPYT44<br>8 | VILLA JUAREZ<br>F2009/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/WBLL1*2/<br>BRAMBLING/5/BAJ #1/AKURI                                 | CMSS11B01152<br>S-099M-099Y-<br>6M-0WGY  | MXI15-<br>16\M7THHPY<br>T\167 |
| 49 | 7HPYT44<br>9 | VILLA JUAREZ<br>F2009/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/WBLL1*2/<br>BRAMBLING/5/ATTILA*2/PB<br>W65//MUU #1/3/FRANCOLIN<br>#1 | CMSS11B01153<br>S-099M-099Y-<br>14M-0WGY | MXI15-<br>16\M7THHPY<br>T\169 |
| 50 | 7HPYT45<br>0 | 68.111/RGB-<br>U//WARD/3/AE.SQUARROSA<br>(321)/4/INQALAB<br>91*2/KUKUNA/5/PBW343*2/K<br>UKUNA/6/MUCUY  | CMSS11B01182<br>S-099M-099Y-<br>8M-0WGY  | MXI15-<br>16\M7THHPY<br>T\170 |

**Annex 2: Genotypic details of 8HPYT (2017/18)**

| E. N. | Name of entries         | Cross Name                   | Selection History   | Origin                        |
|-------|-------------------------|------------------------------|---|-------------------------------|
| 1     | GAUTAM<br>(Local Check) | SIDDHARTH/NING8319/NL2<br>97 | -   | Nepal                         |
| 2     | BAJ #1                  | BAJ #1                       | CGSS01Y00134S-<br>099Y-099M-<br>099M-13Y-0B                 | MXI16-<br>17\M8THH<br>PYT\125 |
| 3     | KACHU<br>#1             | KACHU #1                     | CMSS97M03912T<br>-040Y-020Y-<br>030M-020Y-<br>040M-4Y-2M-0Y | MXI16-<br>17\M8THH<br>PYT\25  |
| 4     | 8HPYT40<br>4            | ZINCSEKTHI                   | CMSA06M00195<br>T-099Y-099Y-<br>9M-0Y-7B-0Y                 | MXI16-<br>17\M8THH<br>PYT\75  |

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| 5  | 8HPYT40<br>5 | MAYIL  | CMSS08Y01129T<br>-099M-099Y-3M-<br>0Y-5M-0Y | MXI16-<br>17\M8THH<br>PYT\100 |
| 6  | 8HPYT40<br>6 | DANPHE<br>#1*2/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//SHA4/CHIL/4/BOKOT<br>A   | CMSS12B01158S-<br>099M-099Y-8M-<br>0WGY     | MXI16-<br>17\M8THH<br>PYT\1   |
| 7  | 8HPYT40<br>7 | QUAIU #1/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/QUAIU<br>#2/5/VILLA JUAREZ<br>F2009/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/WBLL1*<br>2/BRAMBLING   | CMSS12B01180S-<br>099M-099Y-2M-<br>0WGY     | MXI16-<br>17\M8THH<br>PYT\7   |
| 8  | 8HPYT40<br>8 | QUAIU #1/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/QUAIU<br>#2/5/VILLA JUAREZ<br>F2009/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/WBLL1*<br>2/BRAMBLING   | CMSS12B01180S-<br>099M-099Y-32M-<br>0WGY    | MXI16-<br>17\M8THH<br>PYT\8   |
| 9  | 8HPYT40<br>9 | WHEAR/KUKUNA/3/C80.1/3<br>*BATAVIA//2*WBLL1/4/T.D<br>ICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/5/WHEAR/<br>KUKUNA/3/C80.1/3*BATAV<br>IA//2*WBLL1/6/QUAIU<br>#1/SOLALA//QUAIU #2 | CMSS12B01199S-<br>099M-099Y-13M-<br>0WGY    | MXI16-<br>17\M8THH<br>PYT\12  |
| 10 | 8HPYT41<br>0 | MANKU/ZINCOL   | CMSS12B01216S-<br>099M-099Y-6M-<br>0WGY     | MXI16-<br>17\M8THH<br>PYT\15  |
| 11 | 8HPYT41<br>1 | MANKU/ZINCOL   | CMSS12B01216S-<br>099M-099Y-35M-<br>0WGY    | MXI16-<br>17\M8THH<br>PYT\16  |
| 12 | 8HPYT41<br>2 | KOKILA/BOKOTA  | CMSS12B01232S-<br>099M-099Y-12M-<br>0WGY    | MXI16-<br>17\M8THH<br>PYT\21  |
| 13 | 8HPYT41<br>3 | ZINCOL/VALI  | CMSS12B01234S-<br>099M-099Y-10M-<br>0WGY    | MXI16-<br>17\M8THH<br>PYT\24  |

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| 14 | 8HPYT41<br>4 | ZINCOL/VALI  | CMSS12B01234S-099M-099Y-29M-0WGY         | MXI16-17\M8THH<br>PYT\26 |
| 15 | 8HPYT41<br>5 | PAURAQ//RL6043/4*NAC/3/<br>QUAIU #1/SOLALA//QUAIU<br>#2  | CMSS12B01290S-099M-099Y-10M-0WGY         | MXI16-17\M8THH<br>PYT\37 |
| 16 | 8HPYT41<br>6 | DANPHE<br>#1*2/3/T.DICOCCON<br>PI94625/AE.SUARROSA<br>(372)//SHA4/CHIL/4/SHAKTI<br>/5/VALI   | CMSS12B01359T-099TOPY-099M-099Y-32M-0WGY | MXI16-17\M8THH<br>PYT\45 |
| 17 | 8HPYT41<br>7 | VALI*2/6/WHEAR/KUKUN<br>A/3/C80.1/3*BATAVIA//2*W<br>BLL1/4/T.DICOCCON<br>PI94625/AE.SUARROSA<br>(372)//SHA4/CHIL/5/WHEAR<br>/KUKUNA/3/C80.1/3*BATA<br>VIA//2*WBLL1     | CMSS12B01362T-099TOPY-099M-099Y-56M-0WGY | MXI16-17\M8THH<br>PYT\46 |
| 18 | 8HPYT41<br>8 | VALI/6/2*WHEAR/KUKUN<br>A/3/C80.1/3*BATAVIA//2*W<br>BLL1/4/T.DICOCCON<br>PI94625/AE.SUARROSA<br>(372)//SHA4/CHIL/5/WHEAR<br>/KUKUNA/3/C80.1/3*BATA<br>VIA//2*WBLL1     | CMSS12B01363T-099TOPY-099M-099Y-20M-0WGY | MXI16-17\M8THH<br>PYT\47 |
| 19 | 8HPYT41<br>9 | VALI/MAYIL/6/WHEAR/KU<br>KUNA/3/C80.1/3*BATAVIA//<br>2*WBLL1/4/T.DICOCCON<br>PI94625/AE.SUARROSA<br>(372)//SHA4/CHIL/5/WHEAR<br>/KUKUNA/3/C80.1/3*BATA<br>VIA//2*WBLL1 | CMSS12B01366T-099TOPY-099M-099Y-17M-0WGY | MXI16-17\M8THH<br>PYT\51 |
| 20 | 8HPYT42<br>0 | VALI/MAYIL/6/WHEAR/KU<br>KUNA/3/C80.1/3*BATAVIA//<br>2*WBLL1/4/T.DICOCCON<br>PI94625/AE.SUARROSA<br>(372)//SHA4/CHIL/5/WHEAR<br>/KUKUNA/3/C80.1/3*BATA<br>VIA//2*WBLL1 | CMSS12B01366T-099TOPY-099M-099Y-40M-0WGY | MXI16-17\M8THH<br>PYT\53 |
| 21 | 8HPYT42<br>1 | WHEAR/KUKUNA/3/C80.1/3<br>*BATAVIA//2*WBLL1/4/T.D<br>ICOCCON<br>PI94625/AE.SUARROSA<br>(372)//SHA4/CHIL/5/WHEAR  | CMSS12B01368T-099TOPY-099M-099Y-22M-0WGY | MXI16-17\M8THH<br>PYT\54 |

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|    |              | /KUKUNA/3/C80.1/3*BATA<br>VIA//2*WBLL1*2/6/ZINCOL  |  |                               |
| 22 | 8HPYT42<br>2 | COAH90.26.31//KIRITATI/W<br>BLL1/3/KIRITATI/2*WBLL1/<br>6/2*WHEAR/KUKUNA/3/C8<br>0.1/3*BATAVIA//2*WBLL1/4<br>/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//SHA4/CHIL/5/WHEAR<br>/KUKUNA/3/C80.1/3*BATA<br>VIA//2*WBLL1 | CMSS12B01374T<br>-099TOPY-099M-<br>099Y-52M-0WGY | MXI16-<br>17\M8THH<br>PYT\69  |
| 23 | 8HPYT42<br>3 | QUAIU #1/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/QUAIU<br>#2/5/VALI/6/BECARD/QUAI<br>U #1  | CMSS12B01377T<br>-099TOPY-099M-<br>099Y-8M-0WGY  | MXI16-<br>17\M8THH<br>PYT\71  |
| 24 | 8HPYT42<br>4 | QUAIU #1/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/QUAIU<br>#2/5/VALI/6/BECARD/QUAI<br>U #1  | CMSS12B01377T<br>-099TOPY-099M-<br>099Y-10M-0WGY | MXI16-<br>17\M8THH<br>PYT\72  |
| 25 | 8HPYT42<br>5 | MAYIL/ZINCOL//ITP40/AK<br>URI  | CMSS12B01380T<br>-099TOPY-099M-<br>099Y-40M-0WGY | MXI16-<br>17\M8THH<br>PYT\79  |
| 26 | 8HPYT42<br>6 | HOLO/BORL14//VALI  | CMSS12B01392T<br>-099TOPY-099M-<br>099Y-23M-0WGY | MXI16-<br>17\M8THH<br>PYT\84  |
| 27 | 8HPYT42<br>7 | REH/HARE//2*BCN/3/CROC<br>_1/AE.SQUARROSA<br>(213)//PGO/4/HUITES/5/T.SP<br>ELTA<br>PI348599/6/REH/HARE//2*B<br>CN/3/CROC_1/AE.SQUARR<br>OSA<br>(213)//PGO/4/HUITES/7/QUA<br>IU/8/2*QUAIU<br>#1/SOLALA//QUAIU #2        | CMSS12B01406T<br>-099TOPY-099M-<br>099Y-22M-0WGY | MXI16-<br>17\M8THH<br>PYT\94  |
| 28 | 8HPYT42<br>8 | FRET2/TUKURU//FRET2*2/3<br>/T.SPELTA<br>PI348530/4/VALI/5/MUCUY  | CMSS12B01408T<br>-099TOPY-099M-<br>099Y-39M-0WGY | MXI16-<br>17\M8THH<br>PYT\102 |
| 29 | 8HPYT42<br>9 | KATERE/3/QUAIU<br>#1/SOLALA//QUAIU<br>#2/4/BECARD/QUAIU #1   | CMSS12B01418T<br>-099TOPY-099M-<br>099Y-21M-0WGY | MXI16-<br>17\M8THH<br>PYT\105 |

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| 30 | 8HPYT43<br>0 | KATERE/2*BORL14  | CMSS12B01419T<br>-099TOPY-099M-<br>099Y-16M-0WGY | MXI16-<br>17\M8THH<br>PYT\106 |
| 31 | 8HPYT43<br>1 | KATERE/BORL14/3/WBLL1<br>*2/KURUKU//SUP152   | CMSS12B01420T<br>-099TOPY-099M-<br>099Y-18M-0WGY | MXI16-<br>17\M8THH<br>PYT\107 |
| 32 | 8HPYT43<br>2 | KATERE/BORL14/3/WBLL1<br>*2/KURUKU//SUP152   | CMSS12B01420T<br>-099TOPY-099M-<br>099Y-30M-0WGY | MXI16-<br>17\M8THH<br>PYT\110 |
| 33 | 8HPYT43<br>3 | CROC_1/AE.SQUARROSA<br>(210)//PBW343*2/KUKUNA/<br>3/PBW343*2/KUKUNA/4/VA<br>LI/5/MANKU   | CMSS12B01424T<br>-099TOPY-099M-<br>099Y-24M-0WGY | MXI16-<br>17\M8THH<br>PYT\118 |
| 34 | 8HPYT43<br>4 | SHAKTI/2*BORL14  | CMSS12B01430T<br>-099TOPY-099M-<br>099Y-27M-0WGY | MXI16-<br>17\M8THH<br>PYT\120 |
| 35 | 8HPYT43<br>5 | VALI/MAYIL   | CMSS12Y01314S<br>-099Y-099M-<br>099Y-6M-0WGY     | MXI16-<br>17\M8THH<br>PYT\122 |
| 36 | 8HPYT43<br>6 | WHEAR/KUKUNA/3/C80.1/3<br>*BATAVIA//2*WBLL1/4/T.D<br>ICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//SHA4/CHIL/5/WHEAR<br>/KUKUNA/3/C80.1/3*BATA<br>VIA//2*WBLL1/6/DANPHE<br>#1*2/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//SHA4/CHIL | CMSS12Y01317S<br>-099Y-099M-<br>099Y-12M-0WGY    | MXI16-<br>17\M8THH<br>PYT\129 |
| 37 | 8HPYT43<br>7 | WHEAR/KUKUNA/3/C80.1/3<br>*BATAVIA//2*WBLL1/4/T.D<br>ICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//SHA4/CHIL/5/WHEAR<br>/KUKUNA/3/C80.1/3*BATA<br>VIA//2*WBLL1/6/ZINCOL  | CMSS12Y01319S<br>-099Y-099M-<br>099Y-24M-0WGY    | MXI16-<br>17\M8THH<br>PYT\131 |
| 38 | 8HPYT43<br>8 | MAYIL/ZINCOL   | CMSS12Y01376S<br>-099Y-099M-<br>099Y-12M-0WGY    | MXI16-<br>17\M8THH<br>PYT\146 |
| 39 | 8HPYT43<br>9 | HOLO/VALI  | CMSS12Y01405S<br>-099Y-099M-<br>099Y-27M-0WGY    | MXI16-<br>17\M8THH<br>PYT\154 |

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| 40 | 8HPYT44<br>0 | VILLA JUAREZ<br>F2009/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/WBLL1*<br>2/BRAMBLING/5/VALI   | CMSS12Y01415S<br>-099Y-099M-<br>099Y-6M-0WGY              | MXI16-<br>17\M8THH<br>PYT\155 |
| 41 | 8HPYT44<br>1 | VILLA JUAREZ<br>F2009/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/WBLL1*<br>2/BRAMBLING/5/VALI   | CMSS12Y01415S<br>-099Y-099M-<br>099Y-12M-0WGY             | MXI16-<br>17\M8THH<br>PYT\157 |
| 42 | 8HPYT44<br>2 | ZINCOL/3/QUAIU<br>#1/SOLALA//QUAIU #2  | CMSS12Y01432S<br>-099Y-099M-<br>099Y-15M-0WGY             | MXI16-<br>17\M8THH<br>PYT\162 |
| 43 | 8HPYT44<br>3 | REH/HARE//2*BCN/3/CROC<br>_1/AE.SQUARROSA<br>(213)//PGO/4/HUITES/5/T.SP<br>ELTA<br>PI348599/6/REH/HARE//2*B<br>CN/3/CROC_1/AE.SQUARR<br>OSA<br>(213)//PGO/4/HUITES/7/QUA<br>IU/8/KFA/2*KACHU | CMSS12Y01444S<br>-099Y-099M-<br>099Y-33M-0WGY             | MXI16-<br>17\M8THH<br>PYT\165 |
| 44 | 8HPYT44<br>4 | CROC_1/AE.SQUARROSA<br>(210)//PBW343*2/KHVAKI/3/<br>PBW343*2/KUKUNA/4/VAL<br>I   | CMSS12Y01479S<br>-099Y-099M-<br>099Y-34M-0WGY             | MXI16-<br>17\M8THH<br>PYT\167 |
| 45 | 8HPYT44<br>5 | QUAIU #1/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/QUAIU<br>#2/5/CHONTE*2/3/T.DICOC<br>CON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/6/VALI                                 | CMSS12Y01494T<br>-099TOPM-099Y-<br>099M-099Y-11M-<br>0WGY | MXI16-<br>17\M8THH<br>PYT\168 |
| 46 | 8HPYT44<br>6 | QUAIU #1/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/QUAIU<br>#2/5/CHONTE*2/3/T.DICOC<br>CON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/6/VALI                                 | CMSS12Y01494T<br>-099TOPM-099Y-<br>099M-099Y-14M-<br>0WGY | MXI16-<br>17\M8THH<br>PYT\170 |
| 47 | 8HPYT44<br>7 | QUAIU #1/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/QUAIU<br>#2/5/CHONTE*2/3/T.DICOC  | CMSS12Y01494T<br>-099TOPM-099Y-<br>099M-099Y-30M-<br>0WGY | MXI16-<br>17\M8THH<br>PYT\173 |

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|    |              | CON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/6/VALI  |   |                               |
| 48 | 8HPYT44<br>8 | HGO94.7.1.12/2*QUAIU<br>#1/6/CHIH95.2.6/4/BABAX/L<br>R42//BABAX*2/3/SHAMA/5/<br>2*BABAX/LR42//BABAX*2/<br>3/TUKURU/7/SUP152  | CMSS12Y01554T<br>-099TOPM-099Y-<br>099M-099Y-36M-<br>0WGY | MXI16-<br>17\M8THH<br>PYT\192 |
| 49 | 8HPYT44<br>9 | VILLA JUAREZ<br>F2009/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/WBLL1*<br>2/BRAMBLING/5/QUAIU<br>#1/3/T.DICOCCON<br>PI94625/AE.SQUARROSA<br>(372)//3*PASTOR/4/QUAIU<br>#2/6/QUAIU<br>#1/SOLALA//QUAIU #2 | CMSS12Y01570T<br>-099TOPM-099Y-<br>099M-099Y-11M-<br>0WGY | MXI16-<br>17\M8THH<br>PYT\196 |
| 50 | 8HPYT45<br>0 | 68.111/RGB-<br>U//WARD/3/AE.SQUARROS<br>A (321)/4/INQALAB<br>91*2/KUKUNA/5/PBW343*2/<br>KUKUNA/6/MUCUY/7/MAY<br>IL   | CMSS12Y01594T<br>-099TOPM-099Y-<br>099M-099Y-15M-<br>0WGY | MXI16-<br>17\M8THH<br>PYT\198 |