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# PIONEERING NEW FRONTIERS OF MAIZE BREEDING: GENESIS OF A NOVEL HIGH TEMPERATURE STRESS TOLERANT HYBRID FH-988

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## Abstract

Maize productivity is increasingly compromised by changing climatic conditions, with high temperature stress being a major factor in yield losses. Integrated strategies focusing on efficient resource utilization and heat-tolerant maize hybrids are crucial for sustained production. Improving heat tolerance in maize has become one of the top priorities for maize breeding programs. Thus, a new heat-tolerant maize hybrid FH-988 developed in 2021. This hybrid was evaluated in various yield trials from 2011 to 2018 and showed promising yields. It gave 9028 and 9675 kg/ha grain yield in adaptability trials during spring 2017 and 2018 that was 7% higher than its check YH-1898. Under high temperature stress (33.4-43.5ºC) during 2020 & 2021, FH-988 exhibited superior performance against checks DK-6724, YH-1898, P-1429, and FH-1046 by 21%, 13%, 52%, and 49% respectively. Finally, FH-988 not only offers potential as a top choice for the spring season but is also suitable for late sowing.

**Key words:** Hybrid maize breeding, Heat stress, Grain yield.

## Introduction

Maize (*Zea mays* L.) is a major crop in the Pakistan and worldwide. It is preferably chosen by farmers due to its better grain yield/acre among cereal crops and existence of well-established industry. It's a dual-purpose crop and can be used either for fodder or grain. Moreover, it is considered as an industrial crop owing to its heavy usage (70-80%) in industry, whereas only 13% is a part of human consumption, globally (Yousaf *et al*., 2021a). It was cultivated on 205 million hectares and gave 1210 million metric tons production globally in 2021 (Anon., 2021). Maize has diverse utilization all over the world. Corn grain is used for making flour, flakes, syrup, starch and oil extraction (Shehzad *et al*., 2019).

Maize belongs to the C4 group of plants that have the ability to fix $CO_2$ efficiently to produce comparatively higher grain yield (Shehzad *et al*., 2019). Grain yield is an important trait, which is adversely affected by various biotic and abiotic stresses and ultimately raising food security issues (Ghani *et al*., 2020). One of the main challenges for restricted high grain yield in spring season is high temperature stress (HTS). HTS adversely affects grain filling that ultimately decreases grain yield (Yousaf *et al*., 2022). The possible reasons include destruction of chlorophyll (chloroplast), lower light energy absorption and transmission, disrupt electron transfer chain, decreased photosynthetic assimilates, limit photosynthesis, reduce turgor pressure to bring water deficit at grain filling stage. Limited water presence and photosynthesis affected grain filling, causing lower kernel weight and ultimately grain yield (Yousaf *et al*., 2022). Zhi-qiang *et al*., 2016 suggested eight strategies to tolerate or avoid HTS; one of them is breeding of heat tolerant hybrids to gain promised yield.

The current study carries out to share the success story of conventional breeding for development of high temperature stress tolerant hybrid FH-988 by Maize Research Station (MRS), Ayub Agricultural Research Institute (AARI) Faisalabad.

**Plant materials:** Maize Research Station, AARI Faisalabad has developed its own maize germplasm collection by deriving inbred lines from local and exotic maize sources. These inbred lines derivation process was initiated in 1997 and kept continue to develop climate smart maize hybrids.

## Breeding strategy

**Development of inbred lines:** The inbred line F-210 was developed locally through inbreeding (S 0 -S 8) during 2003 -2010 and F-165 during 1997- 2003 from source population available at this Station. F-165 was identified as the best male parent after testing its combining ability (Fig. 1). Derivation material ($S_0$) for developing F-210 Inbred line was sourced from C-17-19 OPV source in 2003 based on high grain rows/cob, tall stature and better performance under heat stress conditions and continued through ear to row selection to derive final inbred line ($S_8$) in 2010 as shown in (Fig. 1).

For maintenance of inbred lines, silk bags were used to cover the ears before silk emergence to avoid unwanted pollen contamination. For pollination, tassel was covered with tassel bags in the evening and pollinated the same plant in the next morning using the pollen containing tassel bag between 8-10 am in spring season.

**Development of FH-988 hybrid:** After the development and evaluation of F-210 and F-165 inbred lines, these were crossed to breed new cross FH-988 in khrif-2010.

## Evaluation of agronomic performance of FH-988 in various yield trials

**Station yield trials, multi-location yield trials and national uniformity yield trial (NUYT):** After the development of FH-988, it underwent successive evaluation: starting with preliminary yield trials (Spring 2011 & 2012) on a 3m² area, followed by micro yield trials (6m²/entry) in Spring 2013 & 2014, and concluding in macro yield trials

(12m²/entry) with three replications. During spring 2016 and 2017, FH-988 was tested in multi-locational yield trials on farmers' fields. Two trials with ten entries each; one contained 6 and the second covered five locations with 200m² area/entry. NUYT studies were conducted for consecutive two spring seasons during 2017 and 2018.

**Distinct uniformity study (DUS):** DUS studies were conducted by FSC & RD Faisalabad of parental inbred lines (F-308, F-165) and hybrid (FH-988) for spring seasons 2017 and 2018 to confirm distinctness, uniformity and stability.

**Evaluation of FH-988 under high temperature stress (HTS):** In 2020 and 2021, grain yield (kg/ha) of FH-988 was assessed under normal and HTS conditions in RCBD with three repeats over four checks. In 2020, normal sowing occurred on February 14th, with delayed sowing taking place on April 21st. In 2021, normal sowing was completed on February 10th, accompanied by late sowing on April 30th. Sowing of maize crop in April month is considered late owing to lethal effects of high temperature stress on grain yield at the time of flowering, pollination and grain filling.

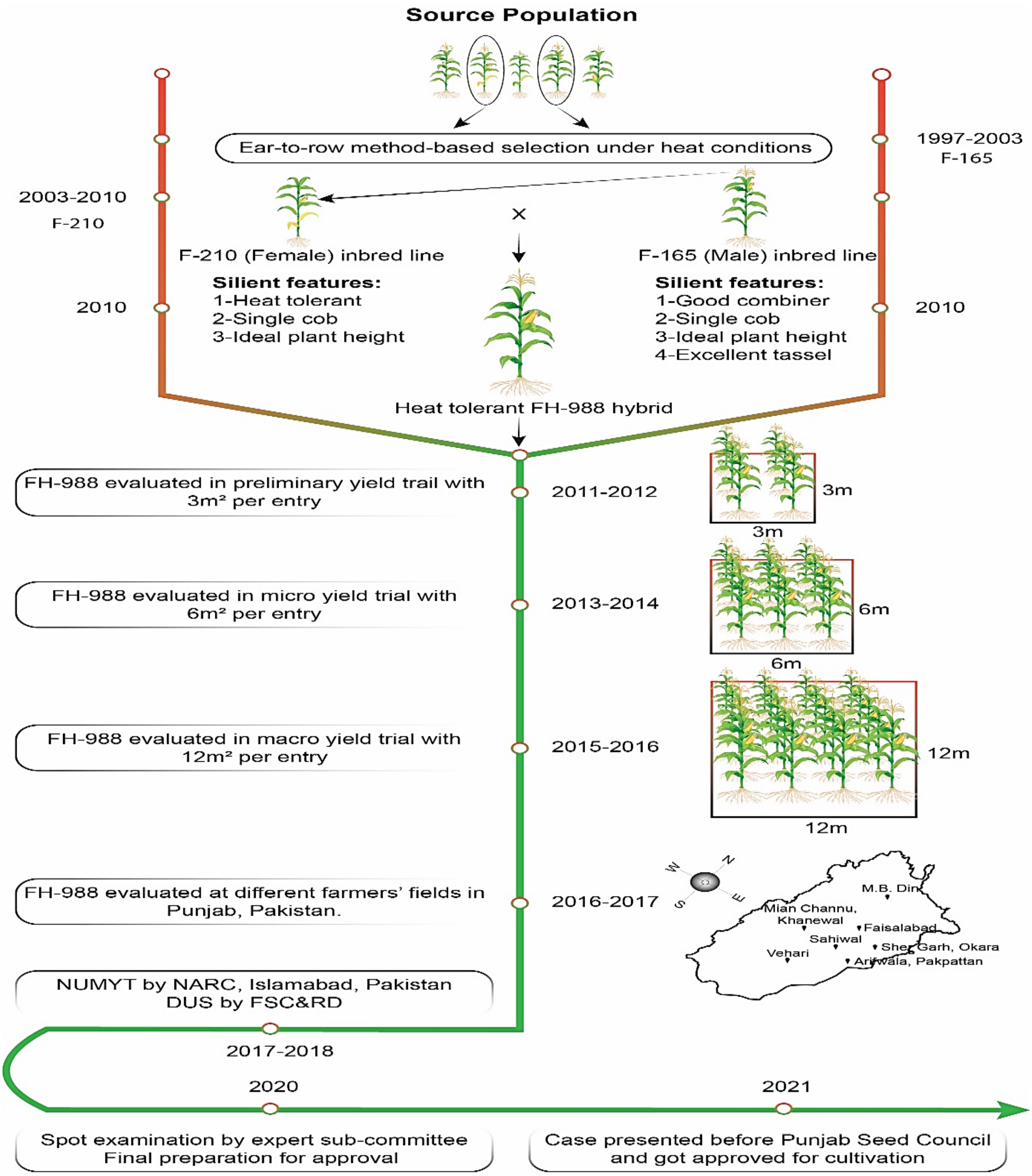

Fig. 1. Breeding History of FH-988 Hybrid.

**Environmental conditions during experimental seasons:** For normal sowing in 2020 and 2021, the recorded ranges of days to 50% tasseling were 66-71 and 65-73 respectively. Under late sowing in 2020 and 2021, 50-56 and 49-54 days to 50% tasseling were observed. Temperature and humidity values are given in (Fig. 2).

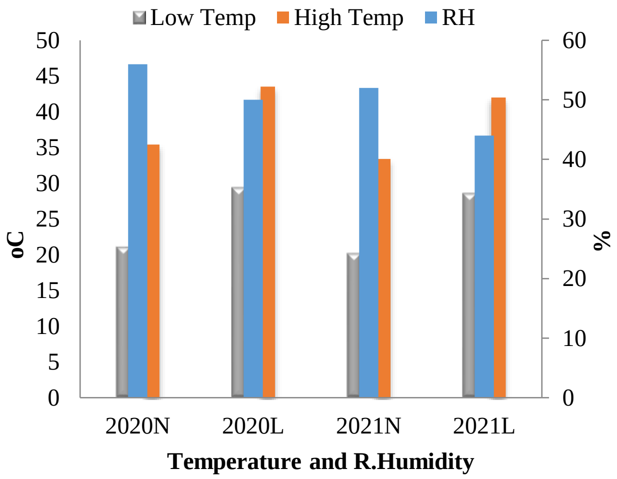

Fig. 2. Average temperature and RH data of 2020 and 2021 for normal and late sowing seasons.

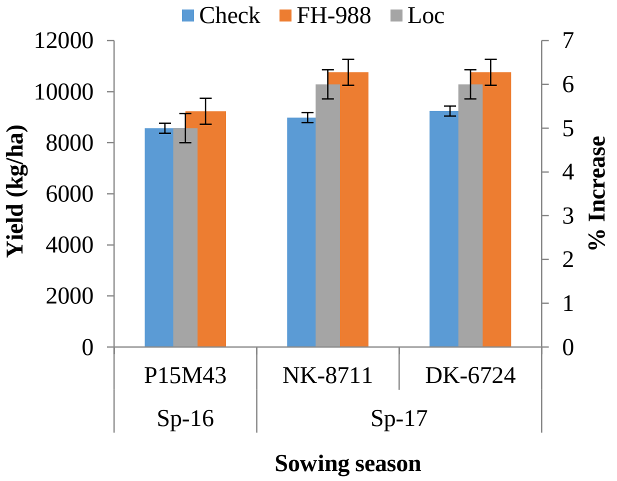

Fig. 4. FH-988 mean grain yield performance in on-farm trials.

**Statistical method:** Combined analysis of variance (Steel *et al*., 1973) of five hybrids for grain yield under normal and HTS conditions for two years (2020 & 2021) were calculated using STATISTIX 8.1 to check significance of differences and their interactions. METAN library of R was used to draw biplots of GEI and biplots were combined in CANVA. MS Excel 2018 was used to draw all graphs.

**Quality parameters:** Quality traits (Crude fat, Crude protein, Ash) were measured through Near Infrared Spectroscopy (NIR) using Inframatic 9200, Partin instruments, Sweden. A sample size of 450g was used for each genotype to estimate the contents of grain quality parameters. For beta carotene compounds, five self-fertilized cobs from each selected genotypes were individually harvested, dried, threshed and bulk seed taken for analysis. The procedure outlined by Kurilich & Juvick, 1999 was used to extract beta carotene from grains.

**DNA finger printing:** For DNA fingerprinting of maize hybrids, seeds of each hybrid and their respective parents were sown in green house at 28°C following the standard agriculture practices. After seedling establishment till 4-5 leaves, 5 seedlings from each genotype were obtained to extract genomic DNA described in the previous studies (Iqbal *et al*., 2021b). The DNA dilutions were prepared to obtain 20 ng/µl concentrations which are required in PCR.

In PCR reaction, 209 SSR markers were selected and applied using the standard procedure described in (Jamil *et al*., 2020b). PCR assembly was followed by Polyacrylamide Gel Electrophoresis (PAGE) for visualization of PCR products and their scoring using the protocol described in (Jamil *et al*., 2021a). The binary data obtained after scoring was subjected to statistical analysis to estimate Polymorphic Information Content (PIC) to estimate the usefulness of different SSR markers.

## Results and Discussion

**Station grain yield trial:** The new hybrid FH-988 performed better to checks in all station yield trials showed in (Fig. 3). FH-988 unveiled maximum grain yield percent increase (33.5) over its check HIC-984 in micro plot yield trial of spring-2013. The minimum percent increase (5.2) was seen in preliminary yield trial of spring 2011 over multinational check NK-8441.

**Out-station grain yield trial:** The average grain yield performance of FH-988 in on-farm yield trials at during spring-2016 remained better (8%) to its check P15M43. The new hybrid also gave satisfactory outcomes in spring-2017 trial over its checks NK-8711 (8%) and DK-6724 (16%) as shown in (Fig. 4).

**National uniform maize hybrid yield trials:** The hybrid FH-988 demonstrated a noteworthy average yield of 9028 kg/ha, surpassing its local competitor YH-1898 by 7% during the year 2017. In 2018, FH-988 exhibited a 7% increment in grain yield compared to its check variety YH-1898, as demonstrated in (Fig. 5).

**Table 1. Analysis of variance (Combined) of yield for five hybrids under normal and HTS conditions.**

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Year (A) | 1 | 51363 | 51363 | 0.11 | 0.752 |
| Condition (B) | 1 | 3.52E+08 | 3.52E+08 | **735.91** | 0.000 |
| A×B | 1 | 602102 | 602102 | 1.26 | 0.294 |
| Rep (C) | | | | | |
| Error A×B×C | 8 | 3824957 | 478120 | | |
| Hybrid (D) | 4 | 1.60E+07 | 4005298 | **10.09** | 0.000 |
| A×D | 4 | 584255 | 146064 | 0.37 | 0.830 |
| B×D | 4 | 8.79E+07 | 2.20E+07 | **55.35** | 0.000 |
| A×B×D | 4 | 462954 | 115738 | 0.29 | 0.881 |
| Error A×B×C×D | 32 | 1.27E+07 | 396893 | | |
| Total | 59 | 4.74E+08 | | | |

Grand mean: 8387, CV (A×B×C): 8.2, CV (A×B×C×D): 7.5

**High temperature stress studies for grain yield:** Combined analysis of variance signifies the effects of conditions (B), hybrid (D) and conditions × hybrids (B×D) interaction as shown in (Table 1). The presence of significant interaction for B×D indicated the influence of both conditions (normal and late sowing) on grain yield of hybrids.

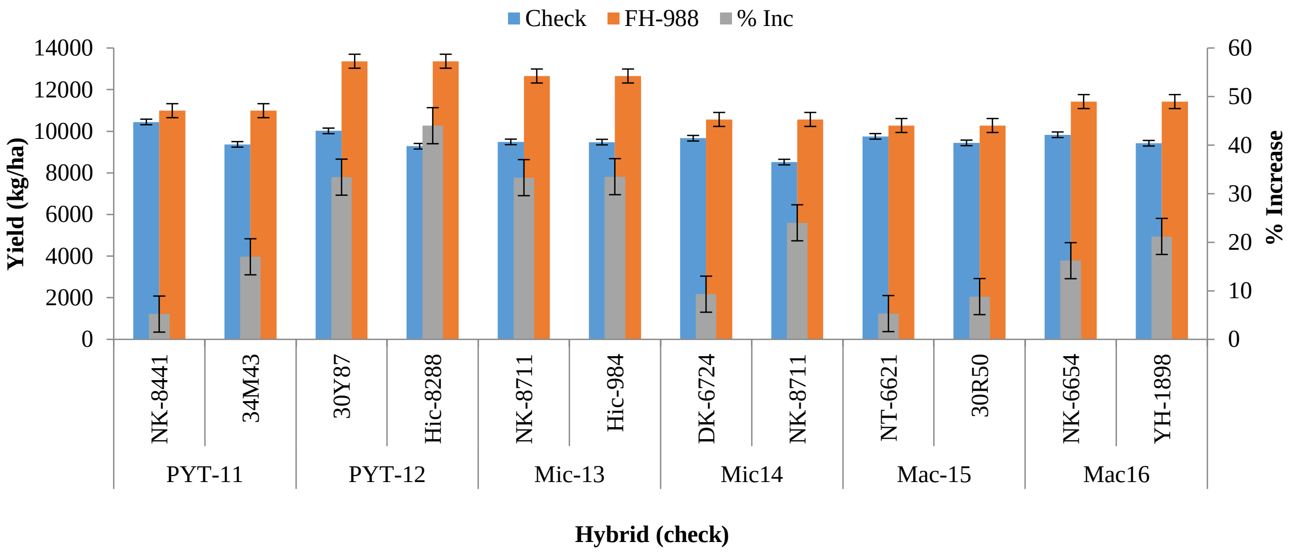

Fig. 3. FH-988 mean grain yield performance in local station trials.

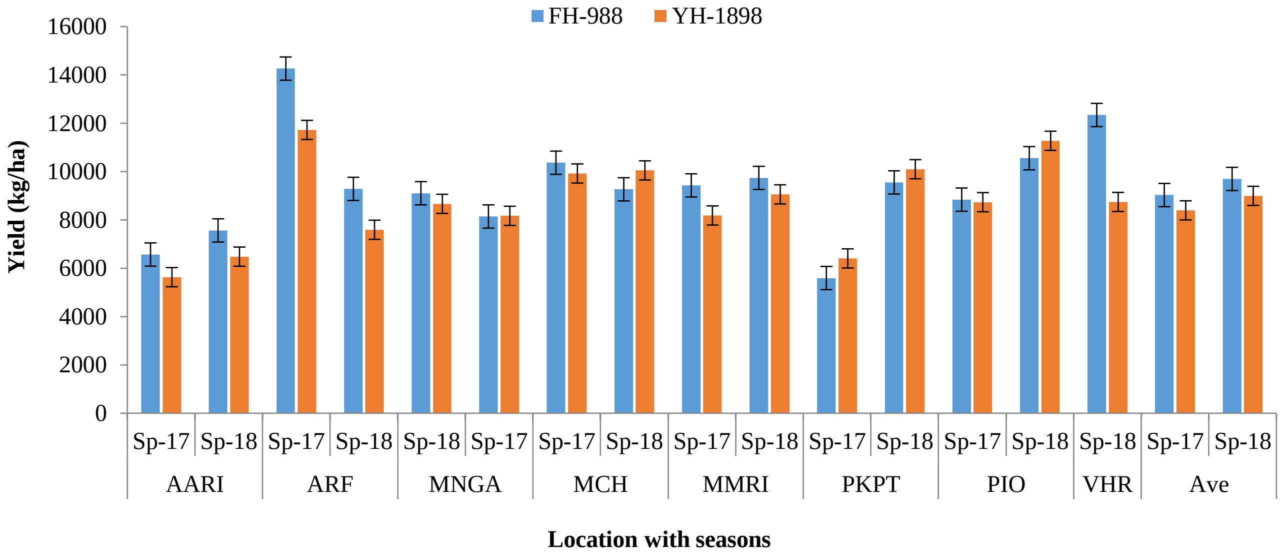

Fig. 5. FH-988 mean grain yield performance in national trials.

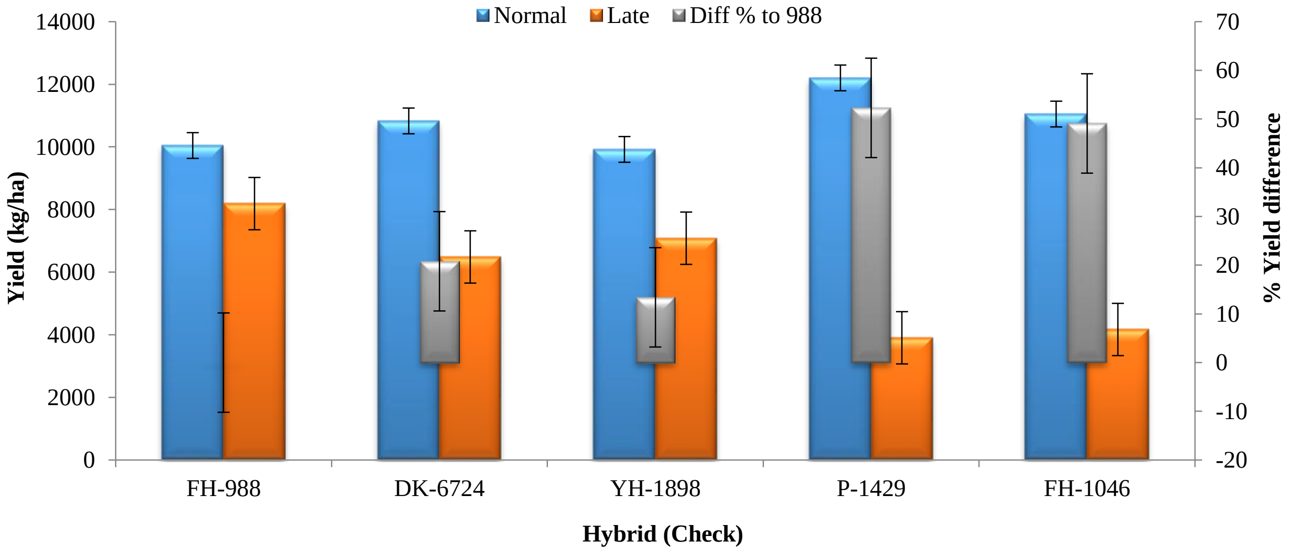

Fig. 6. FH-988 mean grain yield performance under normal and late sowing conditions.

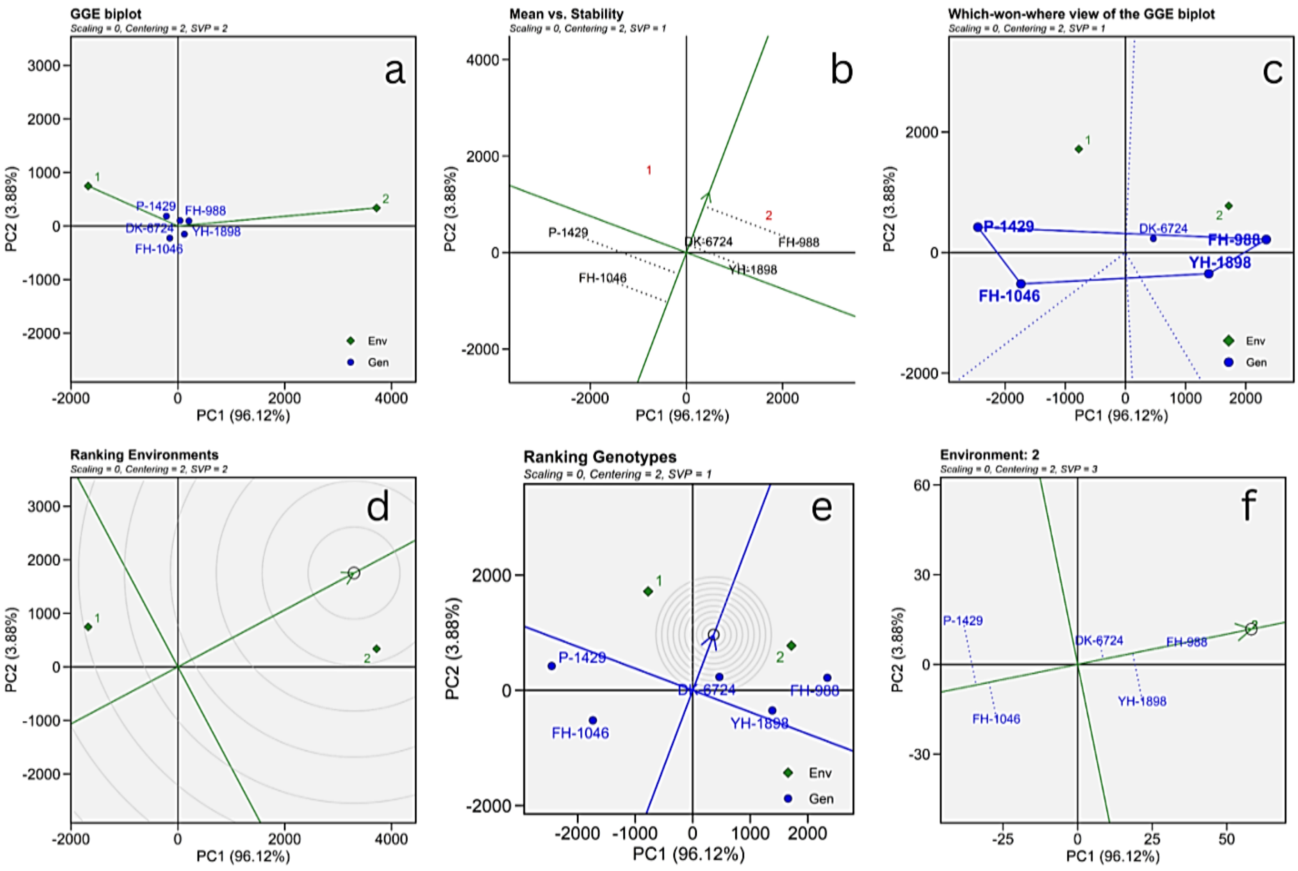

Fig. 7. Patterns (a-f) The relationship between hybrids and environmental tested for yield trait a. GGE biplot b. Mean vs stability c. which won where d. Ranking Env e. Ranking Hybrids. f. Performance in Env 2.

Fig. 6. revealed the mean grain yield of hybrids under two distinct conditions and relative grain yield measure of hybrids from FH-988 under late sowing condition.

Under normal conditions, P-1429 stood out at top position, producing an impressive yield of 12,202 kg/ha, closely followed by FH-1046 (11,049 kg/ha). However, when subjected to high-temperature stress (HTS) in late sowing, the yield dynamics shifted noticeably. The FH-988 hybrid demonstrated resilience with the highest grain yield of 8,188 kg/ha, with YH-1898 trailing behind at 7,083 kg/ha. A comparative analysis revealed that when measured against the grain yield of FH-988 under HTS, YH-1898 saw a 13% reduction, DK-7724 suffered a 20% dip, FH-1046 plummeted by 49%, and notably, P-1429, which was a top performer under normal conditions, experienced a significant 52% drop.

The ultimate objective of a plant breeder is to develop superior varieties/hybrids to gain economic grain yield and breed resistance crop population against biotic and abiotic stresses (Ghani *et al*., 2020). It is found that modern hybrids have higher yield under optimum field conditions but tolerance to various stresses especially to heat stress was lower than land races/older cultivars when treated under high temperature stress (Rafique *et al*., 2020). It was also concluded that landraces that were grown in warmer climate possessed higher tolerance against high temperature stress than grown in cooler region of the world (Ghani *et al*., 2020) consequently; warm climate grown landraces could be used to develop heat tolerant inbred lines and hybrids. It is reported that South Asia production of maize will decrease to 17% if heat and drought stress due to current climate change continues (Shehzad *et al*., 2019; Yousaf *et al*., 2023).

It would be beneficial to breed heat stress tolerance hybrids to secure food availability in future. The newly developed hybrid is highly heat tolerant (44ºC) at reproductive stage and well suited to grow under HTS with minimum loss of grain yield.

The GGE biplots are presented in (Fig 7). Principal component analysis 1 contributed 96.12% and PCA-2 shared 3.88% to the total variation for all biplots (a-f). GGE biplots enabled the researchers to perceive GEI effects and detect the performance of specific genotype for varying environmental conditions (Esan *et al*., 2023).

GGE biplot (a) described the suitability of P-1429 in environment 1 (Normal) while FH-988 in environment 2 (HTS). There exists a marked disparity in the performance outcomes among the various genotypes when analyzed across these two distinct environmental vectors. The genotypes (FH-988, P-1429) that were oriented towards the direction of their environments exhibited superior performance when compared against genotypes (FH-1046, DK-6724, YH-1898) which were positioned behind the origin of the vectors. GGE biplot (b) defined the mean vs. stability of genotypes. The genotypes (FH-988 followed by DK-6724 and YH-1898) that were positioned closer to the arrowhead, demonstrated a performance exceeding the average mean value. The genotypes (DK-6724 followed by FH-1046) with minimal projection from the ordinate of the arrowhead line signifies a higher degree of stability.

GGE biplot (c) indicated which genotype won were. Two mega environments were identified for all genotypes in which P-1429 and FH-1046 showed better performance in 1 mega environment while FH-988, YH-1898 and DK-6724 in 2 mega environments. GGE biplot (d) provides insights into the ideal environmental conditions for the performance of various genotypes. Environment 2 was closer to putative ideal environment (arrowhead circle) followed by environment 1. The proximity of these environments to the arrowhead circle indicates their suitability in fostering ideal conditions for the evaluated genotypes to thrive. GGE biplot (e) identified the ideal genotype by locating inside the circle and closer to the head of arrow in the central part of ring. The only genotype that fell inside the circle is DK-6724. GGE biplot (f) elaborated the best performing genotypes in environment 2. The genotype FH-988 emerges as the top performer, showcasing superior results in this environment. Following FH-988, YH-1898 and DK-6724 are also highlighted for their commendable performance in Environment 2. This sequential arrangement indicates the order of effectiveness, with FH-988 leading the pack, and YH-1898 and DK-6724 subsequently trailing in performance.

## Agronomic studies

**Plant population studies:** Various plant populations per hectare based on varying plant to plant distances were studied during spring-2018 as shown in (Fig. 8). The novel hybrid FH-988 disclosed its maximum yield (12145 kg/ha) at P×P distance of 17.8 cm with 74990 maize plant population per hectare.

**Irrigation trial:** Varying numbers of irrigation were utilized to optimize the yield potential of promising hybrid FH-988 as shown in (Fig. 9).

This hybrid produced maximum yield of 10213 kg/ha at twelve and 10312 kg/ha at thirteen doses of irrigation during spring-2018 season. The results indicate comparable grain yields between two irrigation levels, namely 12 and 13. Consequently, for water conservation purposes, it is recommended to opt for the 12-irrigation level.

**Fertilizer trial:** Various doses of NPK were assessed to uncover the maximum yield potential of the novel hybrid FH-988 during spring-2017 (Fig 10). It evaluated that the best recommended doses of NPK for FH-988 were 306: 113: 74 kg/ha and provided 12105 kg/ha yield.

**Agronomic data:** The promising hybrid FH-988 was evaluated in various experiments to disclose its best suited practices for gaining maximum grain yield potential. (Table 2) shows various recommended practices that can be implemented at the time of sowing.

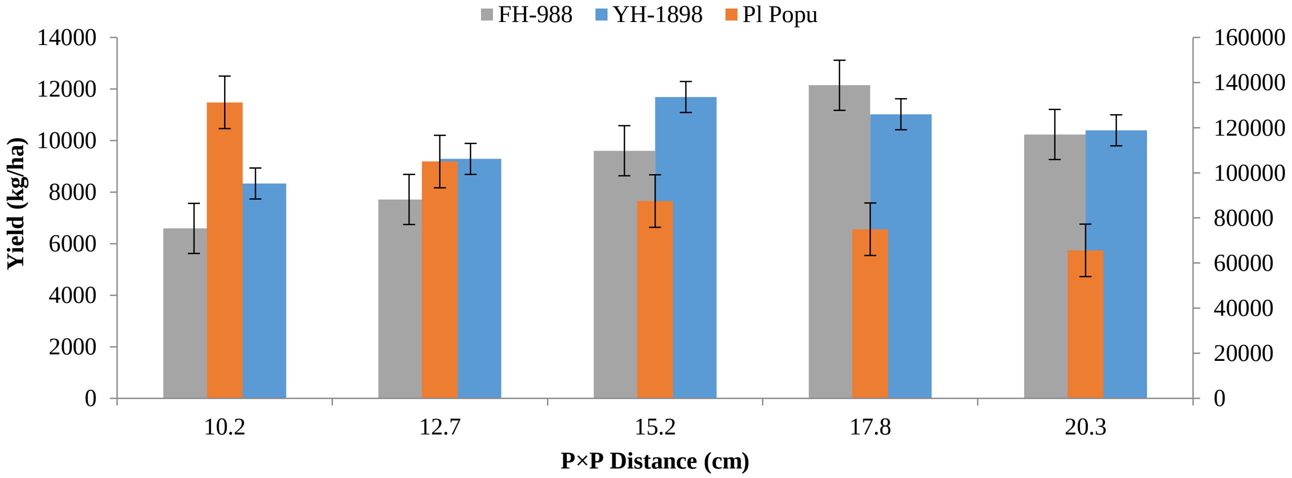

Fig. 8. Grain yield vs plant population.

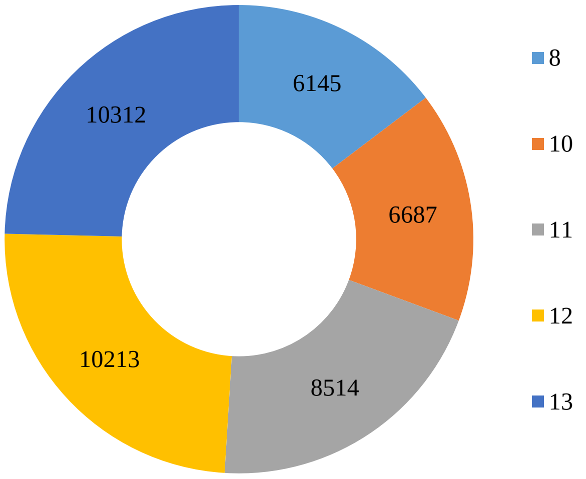

Fig. 9. Grain yield under varying irrigations.

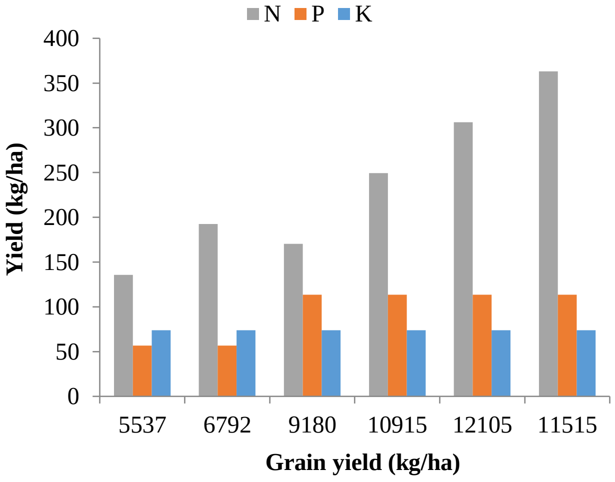

Fig. 10. NPK fertilizers effects on FH-988 grain yield.

**Table 2. Salient Features of FH-988 Hybrid.**

| Practice | Recommendation | Practice | Recommendation |
|---|---|---|---|
| Sowing time | 15 February to 15 March | Row spacing | 76.2 cm (2.5 ft) |
| Sowing method | Ridge sowing | Plant spacing | 17.8 cm |
| Seed rate/ha | 25 kg | Plant population /ha | 74990 |
| Irrigations | 12-13 | Fertilizer (kg/ha) | 306-113-74 PK |

**Table 3. Comparative features descriptions of FH-988, YH-1898 and FH-1046.**

| Features | Plant height (cm) | Ear height (cm) | Days to 50% silk | Leaf color | 1000 GW (gm) | Yield potential (mt/ha) | Shelling % age |
|---|---|---|---|---|---|---|---|
| FH-988 | 230-260 | 110-125 | 78-82 | Green | 310-330 | 14.2 | 84 |
| YH-1898 | 215-225 | 107-114 | 85-87 | Green | 300-310 | 12.4 | 86 |
| FH-1046 | 225-230 | 100-110 | 79-82 | Dark Green | 290-307 | 13.8 | 86 |

**Plant protection studies:** Several local hybrids were evaluated to reveal the resistance potential against fungal disease (stalk rot) through artificial inoculation in 2013, 2015, 2017 spring seasons. The newly developed hybrid FH-988 showed tolerance against stalk (*Fusarium moniliforme*) rot in all evaluated seasons. For entomological studies, Economic threshold levels (ETL) for shoot fly and maize borer are equal to or less than 10%. This hybrid is susceptible to these insects attack depending upon suitable environmental conditions. The damages of these insects can be controlled by application of seed treatment, insecticide spray, granules and biological control.

**Quality aspects:** Three maize hybrids were analyzed for numerous quality characters (Crude fat, Crude protein, Ash and Beta carotene). The newly developed hybrid FH-988 showed 4.28% crude fat that is higher than DK-6724 (3.05%) and lower than NK-8441 (4.46%). FH-988 exhibited 398.5 µg/100gm beta carotene and 9.29% protein that are superior to those of both competing hybrids presented in (Fig. 11).

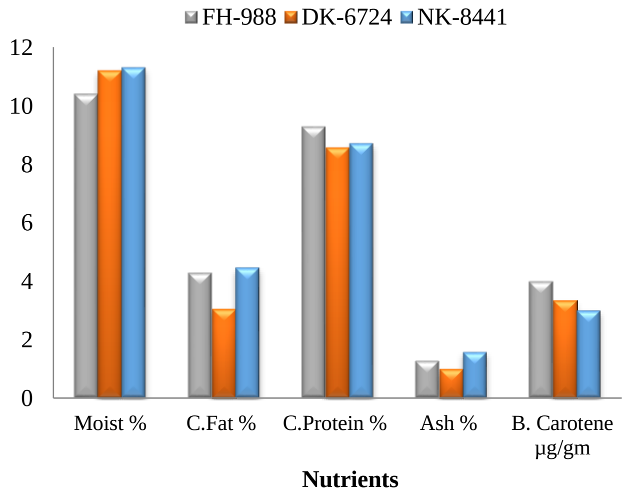

Fig. 11. Nutritional profiling of hybrids.

**DNA finger printing:** Among 209 SSR markers 16 were monomorphic and remaining 193 were polymorphic which amplified a total of 1015 alleles. Maximum number of alleles (26) was amplified by SSR marker (UMC-1676). On an average 5.25 alleles were observed per locus which were very higher than previous reported results (Leggese *et al.*, 2007). Polymorphic information content of the markers was also calculated to study the effectiveness of SSR markers which ranged from 0.1 to 0.67 which are also higher than previous reported results (Pandit *et al.*, 2016).

Cluster analysis was carried out to draw dendrogram based similarity/dissimilarity coefficient using UPGMA algorithm which showed variable genetic similarity 0.54 to 0.961 among maize genotypes (Fig. 12). The candidate hybrid FH-988 varied significantly from the YH-1898, FH-949 and FH-1046 showing a genetic dissimilarity coefficient of 46%. It also varied significantly from other hybrids showing various dissimilarities that showed FH-988 is distinct hybrids from previously registered hybrids and have diverse genetic background (Iqbal *et al.*, 2021a; Jamil *et al.*, 2021a)

**Salient features:** The following prominent traits were recorded for promising hybrid FH-988 in comparison with local approved hybrids YH-1898 (Ghani *et al.*, 2017) and FH-1046 (Rafique *et al.*, 2018) for spring season as revealed in (Table 3).

FH-988 hybrid takes 78-82 days to emerge silk in spring season. It showed 230-260 cm plant height and 110-125 cm ear height that is higher than FH-1046 and YH-1898 as shown in (Table 3). Leaf color remains green after obtaining maturity so can be used for silage purpose. It exhibited 1000 grain weight (310-330 g) which is higher than YH-1898 (300-310g) and FH-1046 (290-307). Shehzad *et al.*, 2019 also presented similar results for 1000 grain weight. Yousaf *et al.*, 2019 and Ghani *et al.*, 2017 presented a positive relationship between 1000 grain weight and grain yield. The yield potential of this hybrid is 14200 kg/ha which is significantly higher than local checks YH-1898 (12400 kg/ha) and FH-1046 (13800 kg/ha). The significant variations in yield among various hybrids were also revealed by Yousaf *et al.*, 2017, Yousaf *et al.*, 2019 and Shehzad *et al.*, 2019.

**Summary:** Newly developed single cross yellow maize hybrid FH-988 stands out as high yielding, spring season, taller and equally suited for grain and silage. One of its significant advantages is its resilience to high temperature stress, a trait that is crucial, especially considering global concerns about climate change and unpredictable weather patterns. This tolerance ensures that the hybrid can thrive and maintain its productivity even under conditions that might be detrimental to other maize genotypes.

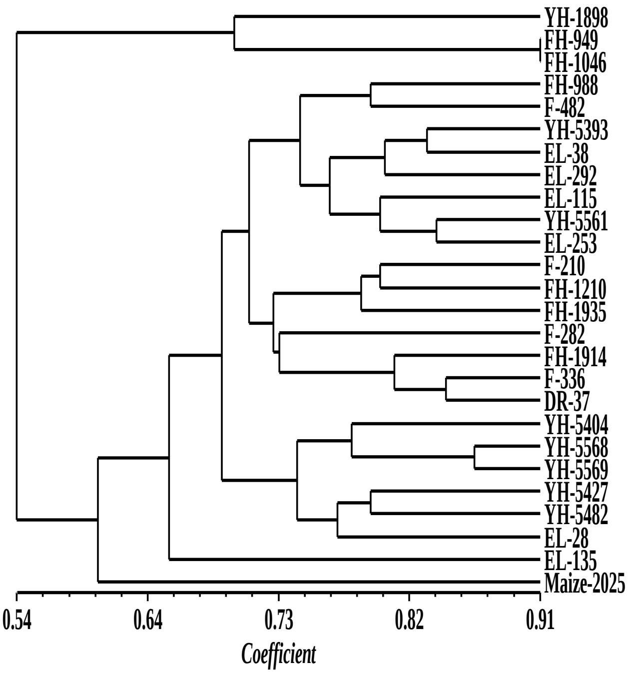

Fig. 12. Dendrogram of 26 maize genotypes constructed based on Jaccords similarity coefficients.

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