



IMPACT OF ENVIRONMENTAL VARIABILITY ON HYBRID NECROSIS IN WHEAT: IMPLICATIONS FOR CLIMATE-RESILIENT BREEDING

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ABSTRACT

Hybrid necrosis, a critical barrier in wheat (*Triticum aestivum* L.) breeding, is significantly influenced by environmental factors, which are increasingly unpredictable due to climate change. This study compares the development of necrosis in 28 wheat hybrids under controlled greenhouse and open-field conditions to understand the role of environmental variability in its expression. The onset of necrosis was earlier in field conditions (12 days post-sowing) compared to the greenhouse (17 days post-sowing), highlighting high expressivity of necrotic combination under low temperature. Morphological traits, including plant height, chlorophyll content, and flowering time, demonstrated marked differences between environments. Greenhouse conditions supported better early growth, while field conditions exacerbated necrosis intensity, leading to stunted growth, delayed flowering, and reduced yield. Only one necrotic hybrid reached the seed development stage in the field compared to five in the greenhouse, reflecting the heightened stress in field environments. These findings underscore the need for breeding strategies that consider the exacerbating effects of climate variability on genetic interactions like hybrid necrosis, which could become more severe under changing climatic conditions.

Key words : Hybrid necrosis, Climate change, Wheat, Environmental stress, Greenhouse, Field conditions.

Introduction

Wheat is widely grown all over the world and stands first among the cereals both in area and production. It has been described as the “King of Cereals” because of the acreage it occupies, high productivity and prominent position it holds in the international food grain trade. It is an important cereal crop of cool climate and plays an important role in food and nutritional security of the world. The exploitation of transgressive segregants which surpass the best parent has been considered a valuable approach in self-pollinating cereals. The transgressive segregants are produced in the F₂ population by accumulation of favorable genes from the parent involved in hybridization.

Hybrid necrosis is a genetic phenomenon in plants where hybrids between two genetically distinct parents exhibit severe growth abnormalities like browning of leaves and stems, stunted development, poor flowering, seed set, and/or premature death. It is caused by complementary dominant genes *Ne1* and *Ne2*, located

on chromosome arms 5BL and 2BS, respectively (Nishikawa *et al.*, 1974; Randhawa *et al.*, 1983). The interaction of specific alleles at the *Ne1* and *Ne2* loci in wheat (*Triticum aestivum*) results in incompatibilities when combined in the hybrid, leading to physiological and developmental defects (Dalal and Khanna-Chopra, 2001; Kumar, 2012). This poses a serious barrier to combining desirable traits from different genotypes of common wheat or transferring genes from related species to commercial cultivars.

The global distribution of these genes reveals that *Ne1* is predominantly found in European and North American wheat varieties, while *Ne2* occurs more frequently in Asian wheat populations, including Indian and Middle Eastern landraces (Tsunewaki, 1960; Kochumadhavan *et al.*, 1984). Historically, hybrid necrosis was first observed when breeders crossed geographically distinct wheat varieties during early 20th-century breeding programs. This phenomenon significantly limited the

integration of desirable traits across diverse wheat populations (Hermsen, 1963).

Crossing between genotypes is essential for gene stacking, which requires manual emasculation and crossing. However, hybrid necrosis can be a significant limiting factor in this process. Understanding the expressivity levels of the genes under different environmental conditions can provide valuable insights, especially in the era of climate change. Even under severe hybrid necrosis, gene stacking and isolating the best combinations are achievable when weather parameters influencing the expression of traits are well understood. Climate change has caused significant shifts in precipitation patterns, temperature fluctuations and the frequency of droughts, all of which directly impact gene expression and distribution (Chu *et al.*, 2006).

The study aimed to evaluate the performance of Parents, N-Hybrids (Necrotic Hybrids) and NN-Hybrids (Non-Necrotic Hybrids) under greenhouse and field conditions to understand their response to variable environments. While necrosis is undesirable, it may occur during gene stacking. Changing the environment can temporarily overcome necrosis to produce seeds, which may segregate in later generations to obtain desirable combinations.

Materials and Methods

The experiment was designed to study hybrid necrosis in wheat, eight wheat genotypes were obtained from the wheat research station, JAU, Junagadh. The crosses were made in a half-diallel fashion to generate the experimental material in rabi 2017-2018. The parent genotypes selected based on literature reviews indicating the presence of *Ne1* and *Ne2* genes. A greenhouse experiment was conducted in the Department of Genetics and Plant Breeding, Junagadh Agricultural University, Junagadh, and a field experiment was conducted at the Instructional Farm, Department of Agronomy, Junagadh Agricultural University, Junagadh, during 2018-2019.

Three parents carrying the *Ne2* gene, three with the *Ne1* gene, and two without necrosis genes were selected based on the review. The details of the selected parents are as follows: GW-496 (*ne1ne1Ne2Ne2*) (Kumar, 2012); GW-366 (*ne1ne1Ne2Ne2*), (Kumar, 2012); GW-322 (*ne1ne1Ne2Ne2*), (Kumar, 2012); Raj-4037 (*ne1ne1ne2ne2*), (Vikas *et al.*, 2013); Raj-4083 (*ne1ne1ne2ne2*), (Vikas *et al.*, 2013); HW-2004 (*Ne1Ne1ne2ne2*), (Vikas *et al.*, 2013); HD-2733 (*Ne1Ne1ne2ne2*), (Vikas *et al.*, 2013); K-9107 (*Ne1Ne1ne2ne2*), (Vikas *et al.*, 2013).

Crosses were developed using hand emasculation and pollination. All possible crosses were created in half diallel fashion. The hybrids (28) along with their parents (8) were screened in green house and field conditions.

In the greenhouse (sowing date: 27/08/2018) experiment was laid in Completely Randomized Design (CRD) with two replications. Sowing was conducted in earthen pots with ten seeds per pot for each treatment. Field (sowing date: 28/11/2018) screening was laid in Randomized Block Design (RBD) with two replications, with each treatment consisting of 25 plants.

The experiment was evaluated for plant height at 30 days after sowing (DAS) and at the harvesting stage. Height was measured from the base to the tip of the main spike (excluding awns) for five randomly selected plants. Chlorophyll Content was measured at 30 DAS using the DMSO method (Almeselmani *et al.*, 2006). Days to 50% flowering was recorded as the number of days from sowing to the flowering of 50% of the plants in each experimental unit. And Biological Yield per Plant was calculated as total biomass produced by the plant. Furthermore, days to necrosis initiation and seed setting was also recorded.

Analysis of Variance (ANOVA) was performed for all the five characters studied and later on Critical difference (CD) and coefficient of variation percent (CV) were calculated. Per se performance of hybrids and parents were presented in the form of line graph and box plot (or box-and-whisker plot). Box Plot is a graphical representation of data distribution based on a five-number summary: minimum, first quartile (Q1), median, third quartile (Q3) and maximum. It highlights the central tendency, spread and presence of outliers in the data.

Results and Discussion

Different expressivity level of necrosis was observed according to the temperature and humidity that prevailed during the developmental period in green house and field conditions. (Dong *et al.*, 2015). In greenhouse conditions, the first necrotic symptoms were observed seventeen days after sowing. In contrast, under field conditions, necrotic symptoms were observed fourteen days after sowing, three days earlier than in greenhouse conditions.

As per review, expected necrotic hybrids should be 16, 17, 18, 26, 27, 28, 36, 37 and 38 (Table 1). But against the expectations 10 necrotic hybrids were obtained *viz.* 16, 17, 26, 27, 36, 37, 46, 47, 68 and 78. The results obtained proved that this is only possible when necrotic gene *Ne2* was present in five parental genotypes—GW-496 (1), GW-366 (2), GW-322 (3), Raj-4037 (4) and K-9107 (5)—instead of three as previously reported (GW-

Table 1 : Expected necrotic hybrids as per review of literature, actual necrotic hybrids and seed setting in parents and hybrids under green house and field conditions.

S. no.	Genotype Code	Expected as per review				Actually Observed				Seed Setting	
		P1	P2	Genotype	Remarks	P1	P2	Genotype	Remarks	Green House	Field
1	1: GW-496	2	2	22	P	2	2	22	P	Yes	Yes
2	2: GW-366	2	2	22	P	2	2	22	P	Yes	Yes
3	3: GW-322	2	2	22	P	2	2	22	P	Yes	Yes
4	4: Raj-4037	0	0	0	P	2	2	22	P	Yes	Yes
5	5: Raj-4083	0	0	0	P	0	0	0	P	Yes	Yes
6	6: HW-2004	1	1	11	P	1	1	11	P	Yes	Yes
7	7: HD-2733	1	1	11	P	1	1	11	P	Yes	Yes
8	8: K-9107	1	1	11	P	2	2	22	P	Yes	Yes
9	12	2	2	22	NNH	2	2	22	NNH	Yes	Yes
10	13	2	2	22	NNH	2	2	22	NNH	Yes	Yes
11	14	2	0	20	NNH	2	2	22	NNH	Yes	Yes
12	15	2	0	20	NNH	2	0	20	NNH	Yes	Yes
13	16	2	1	21	NH	2	1	21	NH	Yes	No
14	17	2	1	21	NH	2	1	21	NH	No	No
15	18	2	1	21	NH	2	2	22	NNH	Yes	Yes
16	23	2	2	22	NNH	2	2	22	NNH	Yes	Yes
17	24	2	0	20	NNH	2	2	22	NNH	Yes	Yes
18	25	2	0	20	NNH	2	0	20	NNH	Yes	Yes
19	26	2	1	21	NH	2	1	21	NH	No	No
20	27	2	1	21	NH	2	1	21	NH	No	No
21	28	2	1	21	NH	2	2	22	NNH	Yes	Yes
22	34	2	0	20	NNH	2	2	22	NNH	Yes	Yes
23	35	2	0	20	NNH	2	0	20	NNH	Yes	Yes
24	36	2	1	21	NH	2	1	21	NH	Yes	No
25	37	2	1	21	NH	2	1	21	NH	No	No
26	38	2	1	21	NH	2	2	22	NNH	Yes	Yes
27	45	0	0	0	NNH	2	0	20	NNH	Yes	Yes
28	46	0	1	1	NNH	2	1	21	NH	Yes	Yes
29	47	0	1	1	NNH	2	1	21	NH	Yes	No
30	48	0	1	1	NNH	2	2	22	NNH	Yes	Yes
31	56	0	1	1	NNH	0	1	1	NNH	Yes	Yes
32	57	0	1	1	NNH	0	1	1	NNH	Yes	Yes
33	58	0	1	1	NNH	0	2	2	NNH	Yes	Yes
34	67	1	1	11	NNH	1	1	11	NNH	Yes	Yes
35	68	1	1	11	NNH	1	2	12	NH	Yes	No
36	78	1	1	11	NNH	1	2	12	NH	No	No

- 1,2,3,4,5,6,7 and 8 indicates genotypes GW-496, GW-366, GW-322, Raj-4037, Raj-4083, HW-2004, HD-2733 and K-9107, respectively.
- Code 12 indicates cross between 1 (female parent GW-496) and 2 (male parent GW-366) and so on for rest of the codes.
- P1 indicates first parent (Female) and P2 indicates second parent (Male)
- In P1 and P2 column “0” indicates recessive non necrotic form of gene, “1” indicates presence of *Ne1* Gene and “2” indicates presence of *Ne2* Gene.
- In genotype column 12 or 21 (*Ne1ne1 Ne2ne2*) genetic combination is necrotic and combinations like 00 (*ne1ne1 ne2ne2*), 01 or 10 (*ne1Ne1ne2ne2*), 22 (*ne1ne1Ne2Ne2*) and 20 or 02 (*ne1ne1 Ne2ne2*) are non-necrotic or normal individuals.
- NH: Necrotic Hybrid, NNH: Non Necrotic Normal Hybrid, P: Normal Parent.



Fig. 1 : Normal wheat plant is shown in figure (A) and Necrotic Wheat Hybrid in plant (B). Black Arrow indicates necrotic leaf.

Table 2 : Analysis of variances (ANOVA) for different characters studied under green house (CRD) and field conditions (RBD) in bread wheat.

S. no.	Characters	Genotype	Replication	Error
CRD experiment under Green House condition				
	df	35	-	36
1.	Plant height at 30 DAS	25.702*	-	4.149
2.	Plant height at harvesting stage	47.070*	-	6.758
3.	Leaf chlorophyll content at 30 DAS	3.758*	-	0.173
4.	Days to 50% flowering	303.152*	-	3.191
5.	Biological yield per plant	2.326*	-	0.046
RBD experiment under Field condition				
	df	35	1	35
6.	Plant height at 30 DAS	19.25*	0.010	1.606
7.	Plant height at harvesting stage	758.220*	247.830*	22.027
8.	Leaf chlorophyll content at 30 DAS	3.050*	0.680	0.218
9.	Days to 50% flowering	66.490*	50.070*	2.074
10.	Biological yield per plant	112.460*	2.140	3.908

Note: *indicates significance at 5% probability.

496, GW-366 and GW-322). Additionally, the *Ne1* gene was found in two parental genotypes—HW-2004 (6) and HD-2733 (7)—instead of three as reported (HW-2004, HD-2733 and K-9107). Similarly, the non-necrotic recessive form of the gene was present in only one parental genotype, Raj-4083, rather than in two (Raj-4037 and Raj-4083) as previously stated. The necrotic symptoms were observed because of complementation between *Ne1* and *Ne2* genes. In Table 1, we can see that the hybrids having 12 or 21 (*Ne1ne1 Ne2ne2*) genetic combination is necrotic and combinations like 00

(*ne1ne1ne2ne2*), 01 or 10 (*ne1Ne1ne2ne2*), 22 (*ne1ne1Ne2Ne2*), 20 or 02 (*ne1ne1Ne2ne2*) and 11 (*Ne1Ne1ne2ne2*) are non-necrotic or normal individuals. Fig. 1 shows the difference between normal individual and necrotic individuals.

The intensity of necrosis varied in both green house and field condition which affected the seed development at maturity. Under green house and field conditions, all eight parents and all non-necrotic hybrids showed seed setting. However, differential response was observed in necrotic hybrids, out of ten, setting was observed in five viz. 16, 36, 46, 47 and 48. Whereas, only one necrotic hybrid “46” was able to reach the maturity to set the seed under field condition.

The earlier onset of symptoms under field conditions might be attributed to fluctuating environmental factors exacerbating *Ne1* and *Ne2* gene interactions (Chu *et al.*, 2006). The unexpected necrotic hybrids suggest variability in gene expressivity, emphasizing hybrid necrosis as a complex genetic system (Khanna-Chopra *et al.*, 1998).

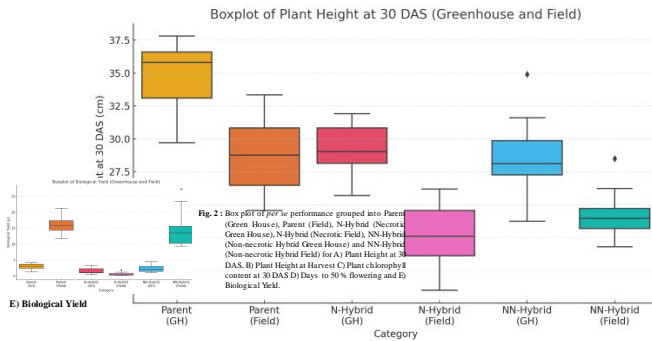
Controlled environments may mitigate necrosis effects, as seen in seed-setting differences between greenhouse and field conditions. However, limited field viability of necrotic hybrids remains a challenge for breeding programs (Hermsen, 1963).

Analysis of variance revealed significance of genotype for all five parameters under both green house and field conditions (Table 2). This indicates that there is difference among the genotypes for the studied characteristics. The purpose of present experiment is to highlight the behavior in performance of different groups viz. parental (Eight), necrotic hybrids (ten) and non-necrotic hybrids (Eighteen) under green house and field conditions. Results of different characters in the form of Box graph is presented and discussed as under

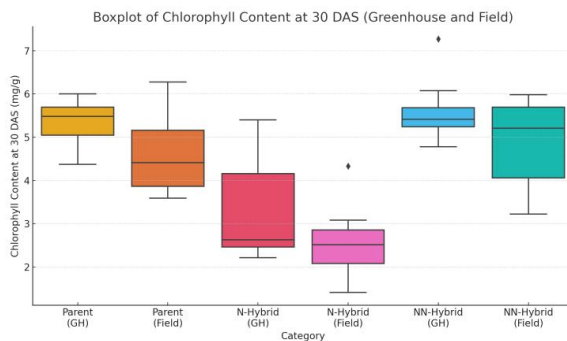
Plant height at 30 Days after sowing (cm)

Looking at the box plot (Fig. 2A) under greenhouse conditions, the median plant height for N-Hybrids was observed to be slightly higher (29.02 cm) compared to NN-Hybrids (28.12 cm). This result, however, can be attributed to the presence of an outlier (34.9 cm) in NN-Hybrids, which influenced their distribution and lowered the median value. The interquartile range (IQR) for both groups was similar, with N-Hybrids having an IQR of 2.67 cm and NN-Hybrids an IQR of 2.61 cm, indicating comparable variability. Parents, on the other hand, exhibited the highest median value (35.8 cm) with a

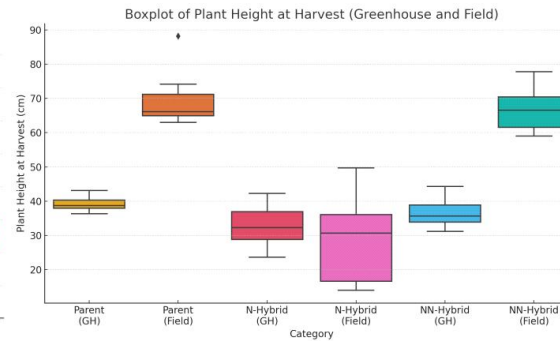
A) Plant Height at 30DAS



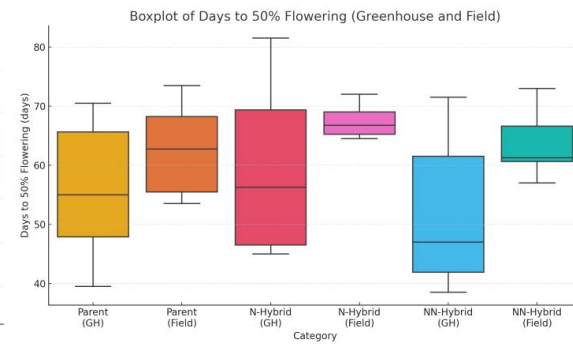
C) Plant Chlorophyll content at 30 DAS



B) Plant Height at Harvest



D) Days to 50 % flowering



narrower IQR, reflecting stable and consistent growth under controlled conditions.

Under field conditions, plant height was reduced across all genotypes, but the differences between categories were more pronounced. Parents maintained the highest median value (28.75 cm) with a slightly broader IQR compared to the greenhouse, reflecting the influence of environmental variability. NN-Hybrids outperformed N-Hybrids in the field, with a median plant height of 24.59 cm compared to 22.6 cm for N-Hybrids. Additionally, the IQR for NN-Hybrids was narrower, highlighting their greater stability under field stress, whereas N-Hybrids exhibited a wider IQR and showed more variability, likely due to the effects of necrosis.

Under field conditions, the detrimental impact of necrosis on N-Hybrids became more evident, as reflected by their significantly lower median plant height and wider IQR. Necrosis symptoms likely impaired physiological processes, reducing growth and causing greater variability among genotypes. In contrast, NN-Hybrids showed better performance, with a higher median and narrower IQR compared to N-Hybrids, suggesting their tolerance to environmental stresses and better genetic stability (Dalal and Khanna-Chopra, 1999). The increase in plant height for all genotypes under greenhouse conditions highlights the etiolated effect.

Plant height at harvest (cm)

The trend was reversed in case of plant height at

harvest in which parents and non-necrotic hybrids exhibited greater plant height as compared to greenhouse conditions. Moreover, field conditions outperformed greenhouse conditions with a significant difference in plant height. However, for necrotic hybrids, plant height remained relatively consistent between field and greenhouse conditions.

Under greenhouse conditions, Parents demonstrated the highest median plant height (38.73 cm) with minimal variability (IQR = 2.40 cm), reflecting their stable performance in controlled environment. NN-Hybrids followed closely with a median of 35.63 cm and a moderately narrow range of performance (IQR = 5.01 cm). In contrast, N-Hybrids showed lower performance with a median plant height of 32.28 cm and greater variability (IQR = 8.13 cm), indicating inconsistent growth even under controlled conditions (Fig. 2 B).

In field conditions, parents maintained their superior performance, exhibiting a median plant height of 66.13 cm (IQR = 6.26 cm), with an outlier highlighting the potential for exceptional genotype performance. NN-Hybrids performed comparably, with a median plant height of 66.53 cm and a slightly higher variability (IQR = 8.89 cm), indicating their adaptability to field stresses. However, N-Hybrids displayed poor growth with a median plant height of 30.7 cm and significant variability (IQR = 19.45 cm), reflecting the pronounced impact of gene expression under lower temperatures (Fig. 2 B).

It is well known that wheat, as a cold loving crop, thrives under prolonged cold conditions. A comparison of temperature and humidity data between the greenhouse and field revealed that the mean field temperature was lower than the greenhouse temperature (Fig. 2 B). For necrotic hybrids, this low temperature proved to be more detrimental than the relatively higher greenhouse temperature (Fig. 3). Furthermore, if we compare plant height at 30 DAS with height at harvest, the greater difference in plant height between necrotic and normal plants (Parents and NN-hybrids) can be attributed to the developmental stage. The peduncle length, a major contributor to plant height, increases rapidly after the boot formation stage under optimal field temperatures. However, in necrotic hybrids, low temperatures intensified gene expression, hindering both the booting and panicle stages (Pan *et al.*, 2017). This early necrosis impaired photosynthetic efficiency, which further contributed to reduce the growth and development and necrotic plants under field conditions.

Chlorophyll content at 30 DAS

Box plotting of the character (Fig. 2C) indicates that

under greenhouse conditions, Parents showed the highest median chlorophyll content (5.48 mg/g) with minimal variability (IQR = 0.65 mg/g), reflecting stable performance. NN-Hybrids exhibited similar performance with a median of 5.46 mg/g and a narrow IQR (0.61 mg/g), indicating stability and uniform chlorophyll retention. In contrast, N-Hybrids recorded a much lower median chlorophyll content (2.63 mg/g) with higher variability (IQR = 1.70 mg/g), suggesting reduced photosynthetic efficiency and inconsistent performance.

Under field conditions, the chlorophyll content decreased across all genotypes. Parents maintained a median of 4.42 mg/g (IQR = 1.30 mg/g), highlighting their stability despite the reduction. NN-Hybrids followed with a median of 5.12 mg/g and a broader IQR (1.77 mg/g), showing slight variability under field conditions but still performing better than N-Hybrids. N-Hybrids exhibited the lowest chlorophyll content (2.52 mg/g) with reduced variability (IQR = 0.77 mg/g), indicating a consistently poor response under stress, likely due to the effects of necrosis.

The results demonstrate that Parents and NN-Hybrids retained higher chlorophyll content and exhibited greater stability under both greenhouse and field conditions. NN-Hybrids, in particular, showed adaptability to field stress, performing close to Parents. In contrast, N-Hybrids recorded significantly lower chlorophyll content, reflecting the impact of necrosis on photosynthetic efficiency.

Days to 50% flowering

Box plot analysis in Fig. 2D indicates that under greenhouse conditions, Parents exhibited earlier and more consistent flowering, with a median of 55 days and moderate variability (IQR = 17.75 days) (Hermsen, 1963). NN-Hybrids showed a slightly earlier median flowering time (50.25 days) but with higher variability (IQR = 20.75 days), indicating some inconsistency among genotypes. In contrast, N-Hybrids displayed delayed and variable flowering, with a median of 56.25 days and the widest variability (IQR = 22.88 days), highlighting the negative impact of necrosis on reproductive development (Dalal and Khanna-Chopra, 1999).

Under field conditions, flowering was delayed across all genotypes, as lower temperatures favored late flowering. Parents maintained their stability, with a median of 62.75 days and reduced variability (IQR = 12.75 days), demonstrating their resilience (Gill *et al.*, 1972). NN-Hybrids followed with a median of 61.25 days and moderate variability (IQR = 8.25 days), suggesting better adaptability to field conditions. N-Hybrids, however, experienced the greatest delay in flowering (median =

66.75 days) with low variability (IQR = 3.75 days), indicating consistently poor performance due to necrosis symptoms (Chu *et al.*, 2006).

The results show that Parents and NN-Hybrids flowered earlier and more consistently under both greenhouse and field conditions. NN-Hybrids demonstrated better adaptability, maintaining flowering closer to that of Parents, particularly under field stress. In contrast, N-Hybrids showed delayed flowering and greater variability in the greenhouse, with consistently late flowering under field conditions, emphasizing the detrimental effects of necrosis. Adjusting environmental conditions can temporarily mitigate necrosis, enabling seed recovery for further generations where segregation may yield desirable trait combinations (Mizuno *et al.*, 2011).

Biological yield

For biological yield it can be clearly seen that all 10 necrotic hybrids performed very poorly under both green house and field condition. But, it is clearly visible that rest of the genotype including parents and non-necrotic hybrids outperformed in field condition (Fig. 2E). Looking at box plot under greenhouse conditions, Parents recorded the highest median biological yield (3.08 g) with minimal variability (IQR = 1.34 g), reflecting their stable performance. NN-Hybrids followed with a median of 2.65 g and a relatively broader IQR (1.64 g), suggesting moderate variability among genotypes. In contrast, N-Hybrids showed the lowest biological yield (1.19 g) with a wider IQR (1.31 g), indicating reduced productivity and inconsistent performance under controlled conditions due to the effects of necrosis.

Under field conditions, Parents maintained their superior performance, with a median yield of 15.76 g and moderate variability (IQR = 2.99 g), highlighting their resilience. NN-Hybrids performed well, with a median yield of 13.88 g and a broader IQR (4.42 g), showing adaptability but with some variability in performance. In contrast, N-Hybrids exhibited extremely low biological yield (0.39 g) with minimal variability (IQR = 0.55 g), reflecting consistently poor performance under field conditions.

The results indicate that Parents and NN-Hybrids achieved higher biological yields under both field conditions. NN-Hybrids demonstrated better adaptability to field stresses, maintaining yield closer to that of Parents. In contrast, N-Hybrids exhibited severely reduced biological yield, particularly under field conditions, due to the adverse effects of necrosis (Dalal and Khanna-Chopra, 1999; Jiang *et al.*, 2008).

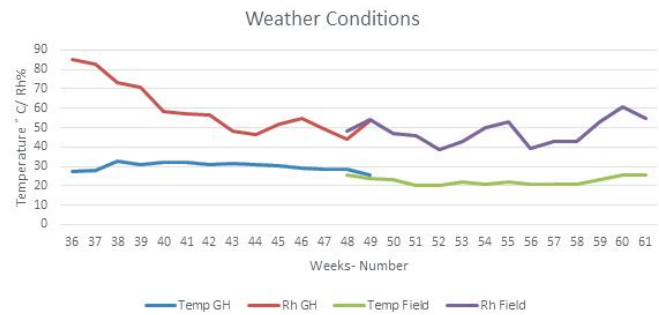


Fig. 3 : Temperature and humidity during the growth period in green house and Field conditions (GH: Green House, Rh: Relative Humidity).

NN-Hybrids demonstrated remarkable adaptability, maintaining performance close to Parents, particularly under field conditions where environmental stresses were more pronounced. This highlights their potential as necrosis-tolerant genotypes suitable for breeding programs aimed at enhancing productivity and stability in variable environments.

In contrast, N-Hybrids were significantly affected by necrosis, displaying reduced performance and greater variability, especially under field conditions. Despite these challenges, the study reaffirms that temporary environmental adjustments, such as greenhouse cultivation, can mitigate necrosis symptoms, enabling the recovery of seeds for further generations for genetic studies and combining the desirable necrotic hybrids.

While necrosis is undesirable, it can occasionally occur during gene stacking. Temporarily altering the environment, such as through greenhouse cultivation, can mitigate its effects to produce seeds. These seeds may segregate in later generations to achieve desirable trait combinations if required. This study underscores the importance of selecting the parental combination during crossing in wheat in which necrotic gene complementation do not takes place.

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References

- Almeselmani, M., Deshmukh P.S., Sairam R.K., Khushwaha S.R. and Singh T.P. (2006). Protective role of antioxidant enzyme under high temperature stress. *Plant Sci.*, **171**, 382-388.
- Chu, C.G., Faris J.D., Friesen T.L. and Xu S.S. (2006). Molecular mapping of hybrid necrosis genes Ne1 and Ne2 in hexaploid wheat using microsatellite markers. *Theoret. Appl. Gen.*, **112**, 1374-1381.
- Dalal, M. and Khanna-Chopra R.K. (1999). Lipid peroxidation is an early event in necrosis of wheat hybrid. *Biochem.*

- Biophys. Res. Commun.*, **262**, 109-112.
- Dalal, M. and Khanna-Chopra R. (2001). Differential response of antioxidant enzymes in leaves of necrotic wheat hybrids and their parents. *Physiologia Plantarum*, **111**(3), 297-304.
- Dong, Y., Wang Y., Xu C., Deng X., Jin Y., Zhang G. and Ma Z. (2015). Transcriptome and proteome analyses of hybrid necrosis in wheat (*Triticum aestivum* L.). *BMC Plant Biology*, **15**, 125.
- Hermesen, J.G. Th. (1963). Hybrid necrosis as a problem for the wheat breeder. *Euphytica*, **12**, 1-120.
- Jiang, Q., Chen H., Pan X., Pan Q., Shi Y., Li X., Zhang G., Wang Y., Xie S. and Shen S. (2008). Proteomic analysis of wheat (*Triticum aestivum* L.) hybrid necrosis. *Plant Science*, **175**, 394-401.
- Jiang, Q., Hu Z., Pan X. and Zhang H. (2013). Comparative proteomic analysis of wheat (*Triticum aestivum* L.) hybrid necrosis. *J. Integ. Agricult.*, **12**(3), 387-397.
- Khanna-Chopra, R.K., Dalal M., Kumar G.P. and Laloraya M. (1998). A genetic system involving superoxide causes F1 necrosis in wheat (*T. aestivum* L.). *Biochem. Biophys. Res. Commun.*, **248**, 712-715.
- Kochumadhavan, M., Tomar S.M.S., Nambisan P.N.N. and Ramanujam S. (1984). Hybrid necrosis and hybrid chlorosis in Indian varieties of *Triticum dicoccum* Schubl. *Euphytica*, **33**, 853-858.
- Kumar, R. (2012). Genetics of hybrid necrosis in wheat (*Triticum aestivum* L.) genotypes. *Unpublished doctoral dissertation*. Junagadh Agricultural University.
- Mizuno, N., Shitsukawa N., Hosogi N., Park P. and Takumi S. (2011). Autoimmune response and repression of mitotic cell division occur in interspecific crosses between tetraploid wheat and *Aegilops tauschii* that show low temperature-induced hybrid necrosis. *The Plant J.*, **68**, 114-128.
- Nishikawa, K., Mori T., Takami N. and Furuta Y. (1974). Mapping of progressive necrosis genes, Ne1 and Ne2, of common wheat by the telocentric method. *Japanese J. Breeding*, **24**, 277-281.
- Pan, S.R., Pan X.L., Pan Q.Y., Shi Y.H., Zhang L., Fan Y. and Xue Y.R. (2017). Ne2 encodes protein(s) and the altered RuBisCO could be the proteomics leader of hybrid necrosis in wheat (*Triticum aestivum* L.). *J. Genetics*, **96**, 261-271.
- Randhawa, A.S., Dhaliwal H.S. and Sharma S.K. (1983). Hybrid necrosis in wheat. *Indian J. Gen. Plant Breed.*, **43**, 370-373.
- Tsunewaki, K. (1960). Necrosis and chlorosis genes in common wheat and its ancestral species. *Seiken Ziho*, **22**, 67-75.
- Vikas, V.K., Tomar S.M.S., Sivasamy M., Kumar J., Jayaprakash P., Kumar A., Peter P., Nisha R. and Punniakotti E. (2013). Hybrid necrosis in wheat: Evolutionary significance or potential barrier for gene flow? *Euphytica*, **194**, 261-275.