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# Novel Technique for precluding Hybrid Necrosis in Bread Wheat

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**Abstract:** *Hybrid necrosis in wheat occurs due to complementary gene action which is expressed only when the responsible genes are brought together in combination. Many adopted varieties of wheat are carriers of these genes, which restricts the parental choice for transfer of desirable traits as the necrotic plants die without producing seeds. In order to overcome the barrier of hybrid necrosis, a study was undertaken to assess the effect of different chemicals for precluding necrosis in bread wheat. To fulfil the purpose, two groups of parents $Ne_2^s$ carriers (S308 and Sonali ) and $Ne_1^s$ carrier (C306) were chosen and made crosses between $Ne_2^s$ and $Ne_1^s$ carrier parents respectively. Their $F_1$s showed strong necrotic expression and died at 3 to 4 leaf stage. During rabi 2009-10, efforts were made to study the effect of proline during gametic fusion or zygotic developmental stage. Two concentrations of proline solution viz; 3 ppm and 5 ppm were prepared and one spray was taken up on the pollinated ears of $Ne_2^s$ and $Ne_1^s$ carriers viz; S 308 x C 306 and Sonali x C306 respectively. Spraying of these two concentrations of proline was done three times during the day period on these two cross combinations S308 x C 306 and Sonali x C 306. When these two hybrids were put for germination in subsequent rabi season 2010-11, 50 normal and 16 necrotic plants were obtained. This study indicates the preclusive role of proline preventing hybrid necrosis in wheat.*

**Key words:** *Complementary gene, Hybrid Necrosis, Proline*

## INTRODUCTION

Hybrid necrosis is a serious problem in wheat breeding because it upsets the crossing programme and put a check on desired parental combinations. It results due to the interaction of two dominant complementary genes $Ne_1$ and $Ne_2$ located on chromosome arms 5BL and 2BS, respectively (Chu *et al.*, 2012). The degree of necrosis varies from severe to moderate and weak. In case of severe necrosis, there is progressive death or debility of green leaf tissue which starts necrotising from tip to the base of first leaf when the hybrid attains 2nd leaf stage. This process continues up to 3 to 4 leaf stage and one by one each leaf is necrotised. In the last, plants die without forming shoots or seeds. Due to occurrence of multiple alleles of $Ne_1^s$ and $Ne_2^s$, the genotypic constitution of $Ne_2^s$ carrier gene parent is expressed as $ne_1ne_1Ne_2^sNe_2^s$ and that of $Ne_1^s$ carrier parent as $Ne_1^sNe_1^s\ ne_2ne_2$ (Hermsen, 1963). Sonalika (S308) and Sonali (HP 1633) carries $Ne_2^s$ gene while C306, HD 2733 and K 8027 carries $Ne_1^s$ gene (Sinha *et al.* 2004). Several approaches such as development of weak-necrotic mutants, bridging necrotic genes through three-way crosses, physical and chemical methods have been made to address the issue of hybrid necrosis (Dhaliwal *et al.*, 1986). Srivastava and Singh (1988) have reported that the development of weak-necrotic mutant variety PNC 306 would be a viable option for preclusion of hybrid necrosis. Sinha *et al.* (2008) have elucidated that the necrotic gene was bridged with non- carrier (HUW 81) and observed some normal plants in $F_1$ generation. Alleviation of hybrid necrosis can be explained based on the fact that the bridged $Ne_1^s$ gene has complemented with the recessive locus of $ne_2$, as a result, hybrid necrosis is not expressed (Sinha *et al.*, 2008). In addition to the above hypotheses, proline has been reported as an

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ameliorating agent of hybrid necrosis (Singh, 2007). However, the effect of proline concentration in preclusion of hybrid necrosis, and method of supplementing studies are limited. As the reports are scanty over the proline alleviator role, a novel study has been undertaken to determine the proline function on hybrid necrosis.

## MATERIALS AND METHODS

During *rabi* 2008-09, two groups of parents $Ne_2^s$ carrier (S308 and Sonali ) and $Ne_1^s$ carrier (C306) were chosen and crosses were made between $Ne_2^s$ and $Ne_1^s$ carrier parents respectively at the experimental Farm of ICAR- Indian Institute of Seed Science, Kushmaur, Mau, U.P. Their $F_1$s showed strong necrotic expression during *rabi* 2009-10 and died at 3 to 4 leaf stage. Therefore, during *rabi* 2009-10, efforts were made to study the effect of proline during gametic fusion or zygotic developmental stage. For the purpose, two concentrations of proline solution viz; 3 ppm and 5 ppm were prepared and one spray was taken up during morning hours in between 8-10 A.M. on the pollinated ears of $Ne_2^s$ and $Ne_1^s$ carriers (S 308 x C 306 and Sonali x C306). Spraying of these two concentrations of proline was done three times during the day period in between 8- 10 A.M., 12-2 P.M. and 4- 6 P.M respectively on the cross combinations of S308 x C 306 and Sonali x C 306 respectively. The spray of proline solution on crossed ears was initiated after third day of pollination and it was regularly maintained up to 10 days. After maturity all the crossed seeds were collected and stored in the descicator.

To validate the spraying effect of proline, seeds of these two hybrids were put for germination in the ICAR- IISS, experimental farm in the subsequent *rabi* season 2010-11. Among all seeds sown, 50 normal and 16 necrotic plants were obtained in the cross combination of S308 x C 306 at 3 ppm concentration of proline sprayed three times in a day. In case of cross combination Sonali x C 306, the number of normal plants was 54 whereas necrotic plants were 17. Similarly, the frequency of normal plants was higher at 5 ppm concentration of proline sprayed three times, in case of both cross combinations (Table 2). The effect of one time spray of both concentrations (3 and 5ppm) of proline solution on the crosses of S308 x C 306 and Sonali x C306 was ineffective because no normal plants were observed.

During *rabi* 2011-12, seeds of all 224 normal plants were sown in the farm plot of ICAR-IISS, Kushmaur, Mau for raising $F_2$ generation. During *rabi* 2011-12, it was observed that 7 weak and 9 moderately necrotic plants were resulted. Rest 90 plants were normal in case of S308 x C 306 cross combination whereas concerning the combination of Sonali x C 306, the number of normal plants was higher (Table 3). Normal plants from both the crosses were selected and their seed samples (500) were sown for rising $F_3$ generation during *rabi* 2012-13. In $F_3$ population, a few weak necrotic plants were noticed.

## RESULTS AND DISCUSSION

*Determination and validation of proline spray effect on hybrid necrosis* – Three times proline sprayed seeds collected during *rabi* 2009-10, were sown to raise $F_1$ generation during *rabi* 2010-11. It was observed that some plants in both the crosses of S 308 x C 306 and Sonali x C 306 were normal and attained maturity (Table 2). Moreover, these hybrids were tall and their ears showed pubescent hairs on the glumes. The presence of pubescent hairs on the glumes of two hybrids S 308 x C 306 and Sonali x C 306 confirmed the sign of true crossing (Narula et al., 1971; Hersman, 1963). Hence, all seeds of such $F_1$ plants were collected to raise $F_2$ generation during the next *rabi* season 2011-12. However, in case of single sprayed proline, hybrids showed strong necrosis and no seed was formed. Earlier studies revealed that the exogenous spray of proline has several applications and concentration dependent. For instance, Gadallah, (1999) has reported that the supplemented proline has mitigated photosynthetic activity reduction under salt stress conditions. Similarly, the result observed of frequent spraying seems to be a concentration dependent. On the other hand, single spray has showed poor effect which may indicate that the concentration may not be enough to ameliorate the preclusion of hybrid lethality (Ahmed *et al.*, 2010).

*Putative role of proline on hybrid necrosis* --- – During *rabi 2010*-11, after three-times spray of 3ppm proline at an interval of 4 h, 50 normal and 16 necrotic plants in the $F_1$s of S 308 x C 306 and 54 normal and 17 necrotic plants in Sonali x C306 have been observed. The frequency of normal plants in three times spray of 5 ppm proline solutions was higher than the 3 ppm concentration in both the cross combinations. Thus, obtaining normal plants elucidates the preclusive role of proline (Fig. 1). However, in case of single spray, no normal plants were obtained in either of the cross combinations. This indicates for preclusion of hybrid necrosis, enough proline concentration is inevitable to overcome the hybrid lethality. These findings are in coherence with the high levels of proline observed in developing seeds of many plants and siliques. Several research studies elucidated the role of proline in embryo development (Székely *et al.*, 2011). From the studies, it has been concluded that mutations in *P5CS2* resulted aborted embryos during late stages of seed development. This corroborates the hypothesis of putative involvement of *P5CS2,* in turn, proline in embryo development. Mattioli *et al.*, (2012) has reported that the alterations of *P5CS2* lead to embryo lethality due to variations in the cellular divisions of aborted embryo. Interestingly, when proline was supplemented exogenously, it triggered meristem formation and organ growth with concomitant expression of cell cycle related protein (CYCB1:1) that indicates the putative role of proline in cell division.

*Assessment of preclusive role of proline in $F_2$ generation* — When $F_2$ generation was raised during *rabi* 2011-12, it was observed that out of 107 plant population, 7 weak and 9 moderately necrotic plants were resulted. The ratio of pubescent: non-pubescent plants were found at a ratio of 3:1 with chi-square value of 0.66 and 0.01 in case of S 308 x C306 and S 308 x Sonali respectively (Table 3). Significant chi-square value indicates that the pubescent trait follow the Mendelian inheritance. When $F_3$ generation was raised during *rabi* 2012-13, only a few weak necrotic plants were observed. This shows the success of proline treatment in hybrid preclusion. It will help in gene flow in desired combinations (Vikas *et al.*, 2013).

**Table 1**
**Pedigree and other details of the varieties**

| *S.N.* | *Variety* | *Pedigree* | *Year of release* | *Production condition* | *Necrotic gene(s) present* |
|---|---|---|---|---|---|
| 1. | S 308 (Sonalika) | II 54.388 /An//Yt 54/N10B/ LR64 | 1968 | Timely and late sown irrigated condition for NWP/NEP zone | $Ne^s_2$ $Ne^s_2$ $ne_1$ $ne_1$ |
| 2. | HP 1633 (Sonali) | RL 6010/5* Sonalika | 1992 | Late sown irrigated condition for NEP zone | $Ne^s_2$ $Ne^s_2$ $ne_1$ $ne_1$ |
| 3. | C 306 | Regent1974/3*Chz//*2C591/ 3/P19/C281 | 1969 | Timely sown rainfed condition for NWP/NEP zone | $Ne^s_1$ $Ne^s_1$ $ne_2$ $ne_2$ |

TS-Timely sown, LS- Late sown, NWP= North Western Plain Zone, NEP= North Eastern Plain Zone

**Table 2**
**Sowing of crossed seeds given spray treatment of different concentrations of proline solution and their performance after germination during *rabi* 2010-11**

| *S.N.* | *Cross combination* | *Proline spray (1 time) on crossed ears* | | | *Proline spray (3 times) on crossed ears* | | |
|---|---|---|---|---|---|---|---|
| | | *Proline conc. ppm)* | *Necrotic plants* | *Normal plants* | *Proline conc.ppm)* | *Necrotic plants* | *Normal plants* |
| 1. | S 308 x C 306 | 3 | 51 | Nil | 3 | 16 | 50 |
| | | 5 | 54 | Nil | 5 | 18 | 57 |
| 2. | Sonali x C 306 | 3 | 51 | Nil | 3 | 17 | 54 |
| | | 5 | 49 | Nil | 5 | 19 | 63 |
| | TOTAL NORMAL PLANTS | | | | | | 224 |

**Table 3**
**Performance of $F_2$ population during *rabi* 2011-12**

| *S.N.* | *Cross combination* | *$F_2$ plants* | *Normal plants* | *Weak necrotic plants* | *Moderate necrotic plants* | *Normal: Necrotic (ratio)* | *Plants with pubescent ears* | *Plants with non-pubescent hairs* | *Ratio Pubescent: Non-pubescent* | *Chi-square value for pubescence* |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | S 308 x C 306 | 107 | 90 | 07 | 09 | 6:1 | 68 | 22 | 3:1 | 0.66 |
| 2. | Sonali x C 306 | 117 | 97 | 09 | 10 | 6:1 | 73 | 24 | 3:1 | 0.01 |
| | TOTAL NORMAL PLANTS | | | | | | | | | 187 |

**Table 4**
**Performance of $F_3$ population during *rabi* 2012-13**

| *S.N.* | *Cross combination* | *Seeds of Normal Plants* | *Segregation for weak/moderate necrosis* |
|---|---|---|---|
| 1. | S 308 x C 306 | 500 | A few weak necrotic plants found |
| 2. | Sonali x C 306 | 500 | A few weak necrotic plants found |

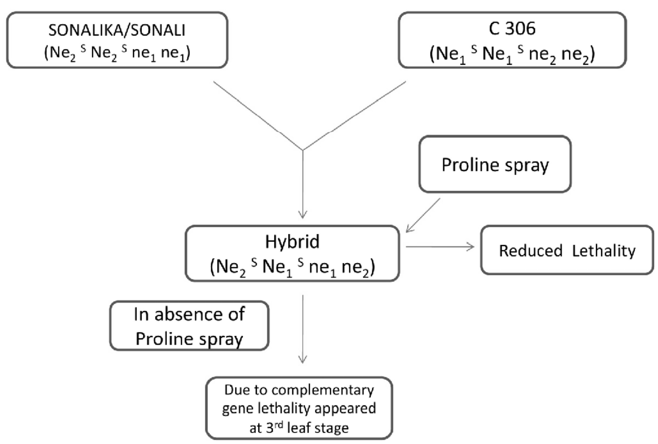


**Figure 1: Schematic diagram of hybrid necrosis and preclusive role of proline**

## CONCLUSION

Hybrid necrosis is a grave concern for the breeders as it hampers the breeding programme of desired combinations. An attempt was made to study the effect of preclusive role of proline by spraying. Proline spray at frequent intervals (three-times-a-day) showed preclusive role and also indicated that the result obtained is concentration dependent. However, single spray has showed poor preclusive role that may be due to lack of enough concentration. Further, the effect was validated in $F_2$ and $F_3$ generation interestingly most of the plants remain precluded. Thus, from the studies it can be concluded that the proline has preclusion effect and has to optimize the concentration and frequency of spraying in wheat.

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