

## Screening physiology of rice drought stress protein

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### ABSTRACT

Achieving drought tolerance in rice requires a deeper understanding of the relationship between possible physiological mechanisms available for water stress tolerance and the identification of expressed proteins under adverse conditions. An investigation was carried out at N.D.U.A.T., Kumarganj, Faizabad. The introgression lines and checks were sown under drought stress and control (irrigated) condition. Drought stress situation was applied 60 days after sowing, while in case of control condition optimum moisture was maintained by frequent irrigations. The rice leaves were selected from drought and control conditions, four introgression lines were selected for 12% acryl amide gel electrophoresis, along with two checks IR 64 and NDR 97. Out of four introgression lines two lines IR 82870-26 and IR 82870-29 were susceptible and other two lines IR 82870-2 and IR 82870-11 were drought tolerant. The leaf protein produced novel bandings patterns in two lines IR 82870-2 and IR 82870-11 rice genotypes which also exhibited good response under drought environment.

**Key words:** drought protein, rice leaf protein, SDS PAGE, leaf rolling scale

The knowledge about the extent and nature of association of rice plant characters among themselves and with yield and yield contributing traits would provide a better understanding in improving yield through selection in field as well as in lab conditions. Drought stress is a major constraint to rice production and yield stability in rained ecosystems (Dey and Upadhyaya, 1996). Rice must be made more drought tolerant, but this is a somewhat contradictory objective considering that rice is most commonly grown under flooded conditions. Achieving drought tolerance in rice will require a deeper understanding of the possible physiological mechanisms available for water stress tolerance and the identification of favourable alleles for introgression into rice varieties that otherwise suit specific environments. Drought stress most severely impacts yield when applied during the reproductive stage of the rice plant. By reducing risk and encouraging farmers to invest in yield-increasing inputs, upland rice cultivars with improved drought resistance could result in greater productivity both in drought years and years with adequate rainfall. Progress in breeding for drought

resistance has been slow (Fukai and Cooper, 1995). When the traits that need to be improved are low in heritability, MAS may be more efficient than phenotypic selection (Asíns, 2002). Drought tolerant near isogenic introgression lines in the genetic background of IR64, developed at the International Rice Research Institute, Philippines (Dwivedi *et al.*, 2004), varieties from geographically diverse localities used in hybridization programme of introgression lines, are available for wide testing and several QTLs for drought tolerance have been identified. In the present study, given introgression lines was evaluated in the stress and control conditions at the Student Instructional Farm of NDUAT, Faizabad.

### MATERIALS AND METHODS

The material for this study consisted of 18 introgression lines developed in the genetic background of IR-64 and three check varieties namely IR 64, Nagina-22 and NDR-97. The introgression lines and checks were sown in same nursery bed. After 25 days single seedling hill<sup>-1</sup> were transplanted with three replications under drought stress and control (irrigated) condition. The crop was

**Table 1.** Mean Performance of Genotypes under Drought Condition

| Entries     | Leaf rolling | Seedling height (cm) | Leaf seedling <sup>-1</sup> | Days to 50% flowering | Plant height (cm) | Panicle bearing tillers plant <sup>-1</sup> | Spikelet panicle <sup>-1</sup> | Grains spikelet <sup>-1</sup> | Spikelet fertility (%) | Test weight (g) | Biological yield (g) | Harvest index (%) | Grain yield (g) |
|-------------|--------------|----------------------|-----------------------------|-----------------------|-------------------|---|--------------------------------|-------------------------------|------------------------|-----------------|----------------------|-------------------|-----------------|
| IR-82870-2  | 0.000        | 23.500               | 4.400                       | 81.333                | 88.133            | 9.400                                       | 111.267                        | 80.000                        | 71.933                 | 17.433          | 35.333               | 43.467            | 15.333          |
| IR-82870-4  | 0.000        | 22.500               | 4.467                       | 74.333                | 82.500            | 9.000                                       | 90.333                         | 71.400                        | 79.167                 | 24.733          | 26.000               | 30.500            | 8.000           |
| IR-82870-6  | 0.333        | 25.033               | 4.533                       | 80.333                | 84.400            | 7.600                                       | 114.400                        | 82.867                        | 72.367                 | 17.533          | 28.000               | 32.267            | 9.000           |
| IR-82870-8  | 0.000        | 19.033               | 3.600                       | 71.000                | 82.433            | 7.400                                       | 114.800                        | 93.733                        | 81.633                 | 19.833          | 33.000               | 33.433            | 11.000          |
| IR-82870-10 | 0.333        | 26.833               | 4.000                       | 78.333                | 90.267            | 8.067                                       | 108.067                        | 84.267                        | 77.967                 | 19.567          | 31.333               | 29.933            | 9.333           |
| IR-82870-11 | 0.000        | 23.000               | 3.733                       | 80.333                | 89.733            | 9.667                                       | 126.267                        | 101.667                       | 80.600                 | 22.400          | 37.333               | 33.800            | 12.667          |
| IR-82870-12 | 0.333        | 27.067               | 3.867                       | 75.000                | 79.367            | 8.200                                       | 86.600                         | 66.267                        | 76.467                 | 25.067          | 26.000               | 28.133            | 7.333           |
| IR-82870-13 | 0.333        | 26.333               | 3.200                       | 77.333                | 96.700            | 6.533                                       | 125.733                        | 106.133                       | 84.433                 | 20.533          | 27.667               | 37.467            | 10.333          |
| IR-82870-14 | 0.333        | 25.800               | 3.467                       | 76.667                | 81.567            | 8.467                                       | 142.667                        | 117.000                       | 82.000                 | 23.467          | 34.333               | 32.833            | 11.333          |
| IR-82870-17 | 0.333        | 24.100               | 3.867                       | 78.667                | 75.800            | 7.800                                       | 94.600                         | 75.800                        | 80.133                 | 19.967          | 27.333               | 28.000            | 7.667           |
| IR-82870-19 | 0.333        | 23.800               | 3.600                       | 78.667                | 81.200            | 6.000                                       | 102.733                        | 83.200                        | 80.967                 | 20.700          | 24.000               | 33.733            | 8.000           |
| IR-82870-21 | 0.000        | 21.933               | 3.267                       | 77.667                | 88.633            | 7.500                                       | 114.667                        | 91.733                        | 80.000                 | 19.100          | 31.667               | 27.333            | 8.667           |
| IR-82870-22 | 1.000        | 26.000               | 3.867                       | 81.333                | 84.633            | 6.800                                       | 109.667                        | 87.667                        | 79.967                 | 17.767          | 25.333               | 31.467            | 8.000           |
| IR-82870-26 | 2.000        | 24.300               | 3.400                       | 81.000                | 82.833            | 5.867                                       | 117.933                        | 88.200                        | 74.833                 | 16.933          | 28.000               | 21.500            | 6.000           |
| IR-82870-29 | 1.000        | 24.633               | 3.200                       | 81.667                | 84.133            | 5.600                                       | 113.667                        | 91.567                        | 80.567                 | 18.567          | 26.000               | 26.967            | 7.000           |
| IR-82870-31 | 1.000        | 24.400               | 3.000                       | 78.667                | 82.933            | 6.400                                       | 107.800                        | 84.933                        | 78.933                 | 17.400          | 24.667               | 35.267            | 8.667           |
| IR-82870-34 | 1.000        | 25.533               | 3.000                       | 78.333                | 84.500            | 8.067                                       | 107.267                        | 90.333                        | 84.167                 | 18.367          | 32.000               | 27.033            | 8.667           |
| IR-82870-35 | 0.333        | 27.367               | 3.000                       | 76.667                | 82.033            | 7.800                                       | 104.733                        | 78.133                        | 74.633                 | 19.500          | 35.667               | 22.533            | 8.000           |
| IR 64       | 0.667        | 18.933               | 4.433                       | 86.000                | 82.033            | 11.600                                      | 114.133                        | 96.867                        | 84.833                 | 19.767          | 41.333               | 38.700            | 16.000          |
| Nageena 22  | 0.333        | 25.167               | 3.000                       | 72.667                | 99.333            | 8.733                                       | 115.533                        | 107.667                       | 93.167                 | 20.267          | 28.667               | 35.067            | 10.000          |
| Narendra 97 | 0.333        | 25.833               | 3.000                       | 71.333                | 76.967            | 10.267                                      | 70.400                         | 59.600                        | 84.667                 | 17.700          | 30.000               | 27.667            | 8.333           |

**Table 1.** Mean Performance of Genotypes under Drought Condition

| Entries     | Seedling height (cm) | Leaf seedling <sup>-1</sup> | Days to 50% flowering | Plant height (cm) | Panicle bearing tillers plant <sup>-1</sup> | Spikelet panicle <sup>-1</sup> | Grains panicle <sup>-1</sup> | Spikelet fertility (%) | Test weight (g) | Biological yield (g) | Harvest index (%) | Grain yield (g) |
|-------------|----------------------|-----------------------------|-----------------------|-------------------|---|--------------------------------|------------------------------|------------------------|-----------------|----------------------|-------------------|-----------------|
| IR-82870-2  | 23.500               | 4.400                       | 81.667                | 87.233            | 8.533                                       | 131.767                        | 110.667                      | 84.133                 | 18.400          | 37.667               | 23.067            | 9.000           |
| IR-82870-4  | 22.500               | 4.467                       | 77.667                | 86.533            | 9.800                                       | 104.467                        | 88.367                       | 84.533                 | 19.367          | 36.000               | 33.167            | 12.000          |
| IR-82870-6  | 25.033               | 4.533                       | 82.333                | 91.667            | 8.000                                       | 144.067                        | 125.333                      | 87.033                 | 24.033          | 38.000               | 29.600            | 13.667          |
| IR-82870-8  | 19.033               | 3.600                       | 72.667                | 89.333            | 8.733                                       | 125.600                        | 115.133                      | 91.767                 | 21.733          | 36.000               | 36.967            | 10.667          |
| IR-82870-10 | 26.833               | 4.200                       | 80.667                | 91.333            | 8.733                                       | 133.733                        | 111.267                      | 83.233                 | 22.300          | 40.000               | 34.967            | 13.000          |
| IR-82870-11 | 23.000               | 3.733                       | 79.000                | 91.767            | 8.133                                       | 149.600                        | 126.067                      | 84.267                 | 24.767          | 28.000               | 40.300            | 12.000          |
| IR-82870-12 | 27.067               | 3.867                       | 77.000                | 89.933            | 8.400                                       | 103.267                        | 84.533                       | 81.900                 | 29.233          | 38.667               | 32.767            | 12.667          |
| IR-82870-13 | 26.333               | 3.200                       | 77.000                | 96.267            | 12.000                                      | 111.333                        | 94.333                       | 84.800                 | 23.033          | 45.000               | 31.167            | 13.000          |
| IR-82870-14 | 25.800               | 3.467                       | 76.667                | 109.60            | 9.000                                       | 159.800                        | 140.067                      | 87.633                 | 29.067          | 48.333               | 31.467            | 16.333          |
| IR-82870-17 | 24.100               | 3.867                       | 80.000                | 86.333            | 9.200                                       | 130.000                        | 109.500                      | 84.433                 | 19.567          | 34.667               | 33.433            | 11.333          |
| IR-82870-19 | 3.800                | 3.600                       | 79.667                | 92.867            | 7.800                                       | 123.200                        | 107.200                      | 87.067                 | 22.400          | 32.000               | 37.400            | 12.000          |
| IR-82870-21 | 21.933               | 3.267                       | 75.667                | 96.567            | 9.267                                       | 117.000                        | 90.733                       | 77.533                 | 22.933          | 31.667               | 33.700            | 10.667          |
| IR-82870-22 | 26.000               | 4.000                       | 83.333                | 82.867            | 7.633                                       | 126.000                        | 106.067                      | 84.133                 | 23.467          | 41.667               | 42.400            | 17.667          |
| IR-82870-26 | 24.300               | 3.400                       | 81.667                | 96.667            | 9.867                                       | 157.400                        | 131.800                      | 83.733                 | 20.200          | 29.333               | 41.600            | 12.000          |
| IR-82870-29 | 24.967               | 3.200                       | 84.333                | 85.600            | 10.133                                      | 124.800                        | 99.333                       | 79.667                 | 17.300          | 28.667               | 49.400            | 14.000          |
| IR-82870-31 | 24.400               | 3.000                       | 81.667                | 86.533            | 8.400                                       | 119.867                        | 100.200                      | 83.733                 | 21.000          | 30.667               | 39.167            | 12.000          |
| IR-82870-34 | 25.533               | 3.000                       | 79.333                | 84.000            | 10.000                                      | 125.933                        | 106.033                      | 84.200                 | 24.567          | 36.000               | 38.833            | 14.000          |
| IR-82870-35 | 7.367                | 3.000                       | 83.667                | 86.633            | 8.733                                       | 125.733                        | 94.467                       | 75.100                 | 21.100          | 32.667               | 34.700            | 11.333          |
| IR 64       | 18.933               | 4.433                       | 80.000                | 89.167            | 9.067                                       | 111.667                        | 92.400                       | 82.767                 | 22.233          | 23.333               | 37.567            | 16.667          |
| Nageena 22  | 25.167               | 3.000                       | 72.000                | 105.00            | 13.067                                      | 96.067                         | 87.000                       | 90.633                 | 20.200          | 40.000               | 36.700            | 14.667          |
| Narendra 97 | 25.833               | 3.000                       | 71.000                | 78.767            | 12.267                                      | 82.733                         | 71.933                       | 86.967                 | 27.600          | 24.000               | 41.433            | 10.000          |

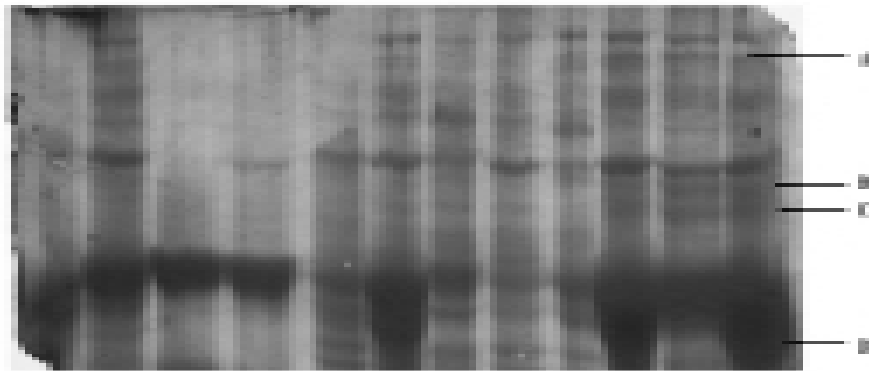


Fig. 2. SDS PAGE gel of all six rice genotypes under drought and control both conditions

| C       | Dt | C     | Dt | C                   | Dt | C           | Dt | C                | Dt | C          | Dt |
|---------|----|-------|----|---------------------|----|-------------|----|------------------|----|------------|----|
| NDR 97  |    | IR 64 |    | IR 82870-29         |    | IR 82870-26 |    | IR 82870-11      |    | IR 82870-2 |    |
| Control |    |       |    | Drought susceptible |    |             |    | Drought Tolerant |    |            |    |

maintained properly at 120:60:60 kg ha<sup>-1</sup> NPK level. The experiment was initially grown under irrigated condition. Rainfed situation was applied after 60 days of sowing in stress conditions, while in case of control condition optimum moisture was maintained by frequent irrigations. Five plants randomly selected from each accession in each replication for recording the observations on seedling vigour, plant height, leaf rolling, days to 50% flowering, panicle bearing tillers plant<sup>-1</sup>, spikelet panicle<sup>-1</sup>, grains panicle<sup>-1</sup>, spikelet fertility, test weight, biological yield, harvest index and grain yield plant<sup>-1</sup>. Crude rice leaf protein isolated by the fresh 5g rice leaf were cut into small pieces and crushed in sodium phosphate buffer (0.25M, pH 7.0) containing 0.15 NaCl. It was homogenized mechanically and centrifuged at 10,000 rpm at 4°C for 20 minutes. This process was done twice. After centrifugation the supernatant was collected. This supernatant was crude rice leaf protein. Protein gel electrophoresis done following the method of Laemmli *et. al.*, (1970).

**RESULT AND DISCUSSION**

Among the high yielding genotypes five most promising genotypes in order of merit were IR 64, IR 82870-2, IR 82870-11, IR 82870-14 and IR 82870-8. IR 64 exhibited good performance for number of leaf, panicle bearing tillers plant<sup>-1</sup>, number of spikelet panicle<sup>-1</sup>, number of grain spikelet<sup>-1</sup>, spikelet fertility, biological

yield and harvest index. IR 82870-2 also exhibited good performance for leaf rolling, seedling height, number of leaf, panicle bearing tillers plant<sup>-1</sup>, spikelet panicle<sup>-1</sup>, number of grains spikelet<sup>-1</sup>, biological yield and harvest index under drought conditions. IR 82870-11 exhibited good performance for leaf rolling, seedling height, panicle bearing tillers plant<sup>-1</sup>, number of spikelet panicle<sup>-1</sup>, number of grains spikelet<sup>-1</sup>, spikelet fertility, test weight and biological yield. Swarna showed better performance for leaf rolling, plant height, panicle bearing tillers plant<sup>-1</sup>, number of spikelet panicle<sup>-1</sup>, number of grains spikelet<sup>-1</sup> and biological yield. Under control condition IR 82870-22 showed highest grain yield among the eighteen introgression lines followed by IR 82870-14, Nagina 22, IR 82870-29 and IR 82870-34. IR 82870-22 exhibited good performance for seedling height, number of leaf seedling<sup>-1</sup>, spikelet panicle<sup>-1</sup>, grains panicle<sup>-1</sup>, spikelet fertility, test weight, biological yield and harvest index.

Out of twenty one lines, rice leaves were selected from both the environments *viz.*, drought and control conditions; four introgression lines (ILs) IR 82870-2, IR 82870-11, IR 82870-26 and IR 82870-29 were selected for protein electrophoresis, along with two checks IR 64 and NDR 97. Out of four ILs two lines IR 82870-26 and IR 82870-29 were susceptible and other two lines IR 82870-2 and IR 82870-11 were drought tolerant as evaluated on 0 to 9 leaf rolling scale

adopting SES, IRRI (Courtois *et al.* 2000) and mean performance of genotypes. The leaf protein of rice genotypes produced novel bandings patterns (A, B, C and D) for a specific protein in two lines IR 82870-2 and IR 82870-11 rice genotypes which also exhibited give good response under drought environment (Salekdeh *et al.*, 2002; Ali and Komatsu, 2006; Swain and Baig, 2008; Ke *et al.*, 2009). These genotypes identified on the basis of desirable mean performance and protein screening may be mentioned as elite lines for their probable genetic worth to be incorporate in hybridization programmes as donor parent for improvement of these characters along with drought tolerance.

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