

The mean values for all traits were compared using the least significance differences test at a 5% level. Five groups of variation were found for LS and DR, four for radicle length, and three for LN and DS (Tables 1 and 2). Length of shoot and dry weight of radi-

Table 1. Average length of shoot (LS), length of radicle (LR) and leaf number (LN) for each genotype.

| LS       |      |        | LR       |      |        | LN       |      |        |
|----------|------|--------|----------|------|--------|----------|------|--------|
| Genotype | Mean | Groups | Genotype | Mean | Groups | Genotype | Mean | Groups |
| 5        | 14.5 | A      | 5        | 18.8 | A      | 16       | 1.2  | A      |
| 6        | 14.0 | AB     | 1        | 18.0 | AB     | 4        | 0.9  | AB     |
| 11       | 13.2 | ABC    | 19       | 16.4 | ABC    | 23       | 0.8  | ABC    |
| 25       | 12.5 | ABCD   | 28       | 16.4 | ABC    | 6        | 0.7  | ABC    |
| 16       | 11.4 | ABCDE  | 6        | 16.4 | ABC    | 22       | 0.7  | ABC    |
| 23       | 11.3 | ABCDE  | 24       | 16.3 | ABC    | 5        | 0.6  | ABC    |
| 29       | 11.2 | ABCDE  | 2        | 16.0 | ABCD   | 8        | 0.6  | ABC    |
| 8        | 10.8 | ABCDE  | 22       | 15.5 | ABCD   | 1        | 0.4  | ABC    |
| 28       | 10.6 | ABCDE  | 13       | 15.1 | ABCD   | 25       | 0.4  | ABC    |
| 10       | 10.6 | ABCDE  | 25       | 14.7 | ABCD   | 10       | 0.3  | BC     |
| 31       | 10.5 | ABCDE  | 29       | 14.6 | ABCD   | 11       | 0.3  | BC     |
| 4        | 10.5 | ABCDE  | 10       | 13.7 | ABCD   | 26       | 0.3  | BC     |
| 13       | 10.4 | ABCDE  | 26       | 13.6 | ABCD   | 24       | 0.2  | BC     |
| 24       | 10.4 | ABCDE  | 23       | 13.6 | ABCD   | 27       | 0.2  | BC     |
| 2        | 8.8  | ABCDE  | 31       | 13.2 | ABCD   | 20       | 0.2  | BC     |
| 1        | 8.8  | ABCDE  | 27       | 13.1 | ABCD   | 31       | 0.2  | BC     |
| 26       | 8.6  | ABCDE  | 18       | 12.9 | ABCD   | 2        | 0.1  | BC     |
| 22       | 8.6  | ABCDE  | 30       | 12.5 | ABCD   | 7        | 0.1  | BC     |
| 19       | 8.2  | ABCDE  | 8        | 12.5 | ABCD   | 13       | 0.1  | BC     |
| 15       | 7.8  | ABCDE  | 11       | 11.8 | ABCD   | 17       | 0.1  | BC     |
| 21       | 7.7  | ABCDE  | 14       | 11.7 | ABCD   | 3        | 0.0  | C      |
| 9        | 7.3  | BCDE   | 4        | 11.0 | ABCD   | 9        | 0.0  | C      |
| 27       | 7.1  | BCDE   | 12       | 10.9 | ABCD   | 12       | 0.0  | C      |
| 20       | 6.8  | CDE    | 20       | 9.9  | ABCD   | 14       | 0.0  | C      |
| 30       | 6.5  | CDE    | 7        | 9.8  | ABCD   | 15       | 0.0  | C      |
| 17       | 6.1  | CDE    | 9        | 9.8  | ABCD   | 18       | 0.0  | C      |
| 18       | 6.0  | DE     | 16       | 9.1  | ABCD   | 19       | 0.0  | C      |
| 14       | 5.6  | DE     | 21       | 7.7  | BCD    | 21       | 0.0  | C      |
| 7        | 5.3  | E      | 3        | 6.7  | CD     | 28       | 0.0  | C      |
| 3        | 4.9  | E      | 15       | 5.5  | D      | 29       | 0.0  | C      |
| 12       | 4.3  | E      | 17       | 5.2  | D      | 30       | 0.0  | C      |

Genotypes with the similar letters are not significantly different at the 5% level

Table 2. Average dry weight of shoot (DS) and dry weight of radicle (DR) for each genotype.

| DS       |       |        | DR       |       |        |
|----------|-------|--------|----------|-------|--------|
| Genotype | Mean  | Groups | Genotype | Mean  | Groups |
| 11       | 544.4 | A      | 4        | 283.9 | A      |
| 6        | 542.4 | A      | 24       | 198.9 | AB     |
| 4        | 528.8 | A      | 31       | 190.6 | ABC    |
| 5        | 494.9 | AB     | 6        | 184.2 | ABC    |
| 24       | 481.9 | ABC    | 28       | 182.9 | ABCD   |
| 29       | 473.5 | ABC    | 14       | 176.8 | ABCD   |
| 28       | 467.7 | ABC    | 8        | 164.0 | ABCDE  |
| 8        | 435.4 | ABC    | 25       | 154.7 | BCDE   |
| 23       | 428.5 | ABC    | 5        | 148.6 | BCDE   |
| 13       | 418.6 | ABC    | 19       | 146.6 | BCDE   |
| 10       | 416.2 | ABC    | 13       | 144.4 | BCDE   |
| 16       | 408.1 | ABC    | 11       | 140.7 | BCDE   |
| 25       | 404.3 | ABC    | 23       | 135.7 | BCDE   |
| 31       | 362.6 | ABC    | 29       | 128.5 | BCDE   |
| 21       | 357.8 | ABC    | 30       | 127.1 | BCDE   |
| 2        | 349.6 | ABC    | 21       | 125.2 | BCDE   |
| 1        | 318.2 | ABC    | 7        | 119.8 | BCDE   |
| 26       | 308.6 | ABC    | 22       | 111.9 | BCDE   |
| 19       | 306.1 | ABC    | 16       | 102.7 | BCDE   |
| 20       | 287.3 | ABC    | 1        | 95.0  | BCDE   |
| 30       | 285.6 | ABC    | 20       | 90.1  | BCDE   |
| 7        | 278.0 | ABC    | 10       | 87.0  | BCDE   |
| 15       | 266.4 | ABC    | 3        | 86.8  | BCDE   |
| 27       | 265.2 | ABC    | 2        | 85.0  | BCDE   |
| 9        | 255.0 | ABC    | 26       | 78.4  | BCDE   |
| 22       | 233.6 | BC     | 27       | 72.7  | CDE    |
| 14       | 214.4 | BC     | 12       | 72.3  | CDE    |
| 12       | 210.1 | BC     | 15       | 69.6  | CDE    |
| 17       | 207.1 | BC     | 9        | 66.8  | CDE    |
| 18       | 203.3 | BC     | 17       | 58.8  | DE     |
| 3        | 197.8 | C      | 18       | 42.5  | E      |

Genotypes with the similar letters are not significantly different at the 5% level

cle were useful in identifying a discriminative response to salinity for the genotypes used.

The technique employed at seedling stages provides a rapid, accurate and inexpensive method for preliminary screening of a large number of accessions. Our results allowed the identification of genotypes with tolerance to saline soils that could be utilized to understand the genetic basis of tolerance and to accelerate a breeding programme in the maize.

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### Additional results from candidate-gene-based association mapping in teosinte

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Our previous association mapping study in teosinte was successful in detecting associations between genetic variation in major regulatory genes in maize and trait variation in teosinte (Weber et al., *Genetics* 177:2349-2359, 2007). Encouraged by this initial success, we have expanded this study by assaying 163 single nucleotide polymorphisms (SNPs) in 68 additional candidate genes in these same individuals. These candidate genes represent genes that have been characterized in maize or genes homologous by sequence to genes characterized in other plant species. We have also included four kernel composition traits (free lysine content, derived starch content, protein content, and oil content) in addition to the 13 traits included in our previous analysis.

Methods were identical to those outlined previously (Weber et al., *Genetics* 177:2349-2359, 2007). As before, not all marker-trait pairs were tested; instead, prior knowledge was used to determine which marker-trait pairs to test. Supplementary materials including the candidate gene list, trait definitions, and a list of marker-trait pairs tested, as well as all of our data files (genotypes, phenotypes, seed source information, principal components and the kinship matrix) are available for download at <http://www.panzea.org>.

Of the 1017 marker-trait pairs tested, only 47 (4.6%) had a *P*-value of less than 0.05, similar to the expectation under the null hypothesis (~5%). Of the 47 detected associations, two withstand correction for multiple testing by the false discovery rate ( $Q < 0.1$ , Table 1). A marker in *waxy1* and a marker in *pbf1* significantly associate with fruitcase weight (FCWT). Both of these associations are biologically plausible given what is known about these candidate genes. *waxy1* is a granule-bound starch synthase that accounts for all amylose production in the kernel (Nelson and Rines, *Biochem. Biophys. Res. Commun.* 9:297-300, 1962; Shure et al., *Cell* 35:225-233, 1983). It is possible that the association of *waxy1* and fruitcase weight is due to its role in amylose production. *pbf1* is a prolamin binding factor hypothesized to be a transcriptional activator of storage proteins in maize (Vicente-Carbajosa et

Table 1. List of significant marker-trait pairs after correction for multiple testing.

| Trait <sup>#</sup> | Gene         | Marker     | N <sup>#</sup> | R <sup>2</sup> | 2a/c <sub>p</sub> | d/a    | P      | FDR Q value |
|--------------------|--------------|------------|----------------|----------------|-------------------|--------|--------|-------------|
| FCWT               | <i>waxy1</i> | PZB00547.3 | 506            | 0.014          | 2.15              | -0.339 | 0.0044 | 0.0770      |
| FCWT               | <i>pbf1</i>  | pbf1.3     | 483            | 0.014          | 0.975             | -0.304 | 0.0077 | 0.0770      |

<sup>#</sup>Number of individuals with both trait and marker data.

al., Proc. Natl. Acad. Sci. USA 94:7685-7690, 1997). Recent work in rice and wheat has found that *pbf1* does act as a transcriptional activator of storage proteins in vivo (Hwang et al., Plant Cell Physiol. 45:1509-1518, 2004). The association between *pbf1* and fruitcase weight could be a result of the role *pbf1* plays in the regulation of storage proteins. Although these associations seem biologically plausible given what is known, further work will be needed to validate that these genes do contribute to natural variation of fruitcase weight in teosinte.

Given the small percentage of associations found to be significant after correction for multiple-testing (< 0.2%), we hypothesize that there are many false negatives among our results. It is likely that our model, which was conservative in regard to controlling the false positive rate due to population structure, led to an increase in the number of false negative associations. We have made our datafiles available on <http://www.panzea.org>, as well as deposited seed from these and other teosinte populations with the U. S. Department of Agriculture North Central Regional Plant Introduction Station in Ames, Iowa, to encourage future teosinte association mapping studies which have the potential to detect genuine biological associations which were not detected in this study.

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### Evidence of interaction between mutants of different *emp* genes

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The symbol *emp* (*empty pericarp*) refers to the phenotype of a group of defective kernel mutants with drastic reduction in endosperm tissue production. Here we report an analysis of the allelic relationship of nine *emp* mutants. They have different origins, thus representing independent mutational events. Originally

they were isolated in populations carrying an active *MuDR* or *Spm* and they all behave as single gene mutants. To establish their allelic relationship we made crosses of each mutant with the others. For each of the pairwise combinations of the nine mutants, pollen from 10-20 plants of a given mutant whose heterozygous condition was ascertained by selfing, was applied to the silks of plants representing the selfed progeny of +/-*emp* parents of a different *emp* isolate. The resulting ears were then scored for visual evidence of mutant segregation. If only wild-type seeds are observed in all ears produced by this cross, the two mutants are not considered to be allelic, whereas if some of the ears yield mutants in about one-quarter of the seeds this is taken as evidence of allelism. Wild-type seeds are then tested further in F2 and F3, the expectation being that ears should be recovered segregating 3 to 1 for the mutant, or not segregating in a 3 to 1 ratio. If the F2 obtained by selfing non-mutant plants of the F1 progeny includes ears segregating an excess of mutants (30-40%), this segregation value, approaching a 9 to 7 ratio, is taken as evidence of heterozygosity for two *emp* mutants in the parental F1 plant, thus defining two genes. The results of these tests, presented in Tables 1 and 2, are generally concordant in their conclusions. In two cases, however, where enough data have been collected, the results obtained in F1 and in F2/F3 lead to contrasting conclusions, i.e., one gene as inferred from the lack of complementation observed in F1, and two genes based on the observation of a segregation close to a 9 to 7 ratio, which is expected when the heterozygous *emp* F1 plants identify two genes.

These intriguing results seem to suggest an interaction between different *emp* mutants. Technically similar events are referred to in the literature as second site non-complementation (SSNC). There are 3 possible explanations for these events: interaction between two different mutant proteins leading to a toxic product, the mutant form of one protein sequestering the wild-type

Table 1. Results of the complementation test involving nine independently isolated *emp* mutants. + and - indicate complementation and non-complementation, respectively. Signs in parentheses refer to dubious results that need further validation.

| ♀↓             | ♂→ | <i>emp4</i> | <i>emp8075</i> | <i>emp8077</i> | <i>emp8300</i> | <i>emp8376</i> | <i>emp8971</i> | <i>emp9106</i> | <i>empDAP3</i> | <i>emp9475</i> |
|----------------|----|-------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| <i>emp4</i>    |    | -           | +              | +              | +              | +              | +              | +              | +              | -              |
| <i>emp8075</i> |    |             | -              | +              | (-)            | (-)            | +              | +              | +              | (-)            |
| <i>emp8077</i> |    |             |                | -              | +              | +              | +              | -              | +              | +              |
| <i>emp8300</i> |    |             |                |                | -              | +              | (-)            | -              | +              | +              |
| <i>emp8376</i> |    |             |                |                |                | -              | +              | +              | (+)            | +              |
| <i>emp8971</i> |    |             |                |                |                |                | -              | +              | (+)            | -              |
| <i>emp9106</i> |    |             |                |                |                |                |                | -              | +              | +              |
| <i>empDAP3</i> |    |             |                |                |                |                |                |                | -              | +              |
| <i>emp9475</i> |    |             |                |                |                |                |                |                |                | -              |

Table 2. Segregation in F2 and F3 of double mutants exhibiting non-complementation in the F1. Signs in parentheses refer to dubious results that need further validation.

| Cross mode               | Non complementation in F1 (# of ears) | Segregation > 30% in F2/F3 (# of ears) | Inferred number of genes from |       |
|--------------------------|---------------------------------------|--|-------------------------------|-------|
|                          |                                       |  | F1                            | F2/F3 |
| <i>emp4 x emp9475</i>    | 9/29                                  | 14/78                                  | 1                             | 2     |
| <i>emp8075 x emp8300</i> | 1/22                                  | 6/27                                   | (1)                           | 2     |
| <i>emp8075 x emp9475</i> | 4/12                                  | -                                      | 1                             | -     |
| <i>emp8077 x emp9106</i> | 14/28                                 | 4/35                                   | 1                             | (2)   |
| <i>emp8300 x emp8971</i> | 1/15                                  | 3/12                                   | (2)                           | 2     |
| <i>emp8300 x emp9106</i> | 9/29                                  | 5/36                                   | 1                             | 2     |
| <i>emp8376 x empDAP3</i> | (1)/13                                | 2/18                                   | 2                             | 2     |
| <i>emp8971 x empDAP3</i> | 2/20                                  | 0/6                                    | 1                             | 1     |
| <i>emp8971 x emp9475</i> | 4/25                                  | 2/67                                   | 1                             | 1     |

form of the other protein into an inactive complex, or combined haplo-insufficiency (Hawley and Gilliland, Genetics 174:5-15, 2006). We will test which of these possibilities applies to the cases reported here.

### Desiccation tolerance of maize viviparous mutants

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In maize, desiccation tolerance is acquired by the embryo at a precise developmental stage between 20 and 25 DAP (days after pollination) and is probably related to the maturation process char-