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.

MILAN, ITALY
Università degli Studi di Milano

Evidence of interaction between mutants of different *emp* genes

--Sangiorgio, S; Gabotti, D; Consonni, G; Gavazzi, G

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 heterozygou *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 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.

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.

♀↓ ♂→	*emp4*	*emp8075*	*emp8077*	*emp8300*	*emp8376*	*emp8971*	*emp9106*	*empDAP3*	*emp9475*
emp4	-	+	+	+	+	+	+	+	-
emp8075		-	+	(-)	(-)	+	+	+	(-)
emp8077			-	+	+	+	-	+	+
emp8300				-	+	(-)	-	+	+
emp8376					-	+	+	(+)	+
emp8971						-	+	(+)	-
emp9106							-	+	+
empDAP3								-	+
emp9475									-

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

Desiccation tolerance of maize viviparous mutants

--Malgioglio, A; Quattrini, E; Della Pina, S; Spini, A; Gavazzi, G

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-

acterized by the accumulation of storage products and LEA (late embryogenesis abundant) proteins with a protective role.

Viviparous mutant embryos that are deficient in ABA synthesis or lack an active *vp1* factor do not express the normal set of maturation phase proteins and should not acquire desiccation tolerance. To verify this, we applied premature desiccation to developing *vp* embryos about 25 DAP, and compared their germination capacity to sib embryos not subjected to such treatment (Durantini et al., Heredity 101:465-470, 2008). To this aim, *vp* and normal sibling embryos from a segregating ear were excised and transferred to plant cell culture vessels (Phytatray Sigma) on basal MS medium containing 2% sucrose solidified with 0.8% agar, or subjected to desiccation and a storage period of 60 days at 5°C before transfer to the same medium. For the drying treatment, mutant and normal embryos were laid between two disks of blotting paper within a sterile petri dish and incubated in an oven at 35°C for 48 hours.

At the end of the treatment, the dishes were sealed with parafilm and conserved at 5°C with silica gel at the bottom of the petri dish under the blotting paper. For the germination test, embryos were maintained in a growth chamber at 25°C with a 14/10 h light/dark photoperiod. Germination was determined after 10 days of culture. When cultured immediately after their excision, immature embryos of all mutants tested germinated with a high frequency (95-100%) like their wild-type counterparts (data not shown). On the other hand, if they were cultured following a premature dehydration treatment, only *vp1* and *vp10* maintained a partial desiccation tolerance while the other mutants lost it (Table 1, Fig. 1). In addition, *vp5* showed minimal germination, consisting of primary root protrusion without a shoot.

In contrast to the results presented in this report, White et al. (Plant Physiol. 122:1081-1088, 2000) showed that induction of GA deficiency early in seed development, either genetically or via biosynthesis inhibitors, suppressed vivipary of *vp5/vp5* mutants while maintaining desiccation tolerance. However, since we applied a different protocol to test desiccation tolerance of the viviparous mutants that did not involve the inhibitors of GA synthesis, the results obtained are not strictly comparable. Furthermore, while interpreting these data one should keep in mind that the nature and position of the molecular lesion within the gene might affect the germination test of the mutant under testing, as clearly shown by the analysis of the alleles of *vp7* (*ps1*) obtained by *Ac* insertional mutagenesis (Bai et al., Genetics 175-981-992, 2007). We also found that *vp10-374* mutants exhibiting partial desiccation tolerance are impaired, to a different extent, in their morphogenesis.

These results seem to suggest that acquisition of desiccation tolerance requires the completion of the steps between carotenoid production and the late stage of ABA biosynthesis, suggesting a link between embryo morphogenesis and desiccation tolerance which should be further investigated.

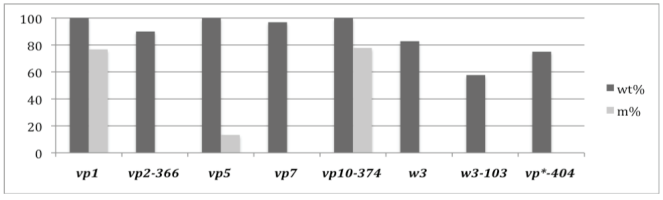

Figure 1. Effect of premature dehydration on germination capacity of wild-type (wt) and viviparous sib embryos.

Table 1. Germination percentage of homozygous *vp* mutant embryos. The germination percentage of the corresponding wild-type sibs is shown in Figure 1.

			+		m	
CODE	DAP	Mutant	No. seeds	germ %	No. seeds	germ %
07.50A-2	26	*vp1*	31	100	30	77
07.01-2	28	*vp2-366*	10	90	9	0
07.62-15	25	*vp5*	30	100	30	13
07.21-2,7	24,26	*vp7*	32	97	30	0
06.182	24	*vp10-374*	24	100	18	78
07.67-7	26	*w3*	29	83	38	0
07.06-1,2	21,24	*w3-103*	33	58	17	0
06.51-N	25	*vp*-404*	20	75	8	0

+ and m refer to wild-type and viviparous siblings

Another case of second site non-complementation

--Galbiati, M; Gavazzi, G

We observed another case of second site non-complementation (SSNC) while analyzing the complementation pattern of *d11**, a dwarf mutant inherited as a monogenic trait. Homozygous *d11** plants, which are drastically reduced in stature, produce andromonecious ears with normal seed set and exhibit a significant increase in their elongation if grown in the presence of 10 µM GA (Galbiati et al., Maydica 47:169-180, 2002). The pattern of complementation of this mutant with recessive *d* mutants reported in the literature (*d1*, *d2*, *d3*, *d5* and *an1*) is unexpected since it indicates that the mutant complements *d3* and *an1* but fails to complement *d1, d2* and *d5*. By further testing each of the double mutants in the F2 generation, we confirmed allelism of *d11** with *d1* and *d5*, a surprising result. On the other hand, the selfed progeny of heterozygous *d11**/+ *d2*/+ dihybrids produceed ears with a 9 to 7 segregation of normal versus dwarfs, a segregation expected if *d2* and *d11** define two separate genes. We hypothesize that the contrasting results observed in the F1 and F2 generation could indicate an interaction of the gene products of two genes as described in our previous report. While germinating seeds of the 9:7 segregating ears, we noticed that a minority of the dwarf seedlings had a more pronounced reduction in their elongation. A similar observation also applied to the F2 progeny of *d11**/+ *an1*/+ parents. In the F2 progeny of these heterozygous double mutants, one-seventh of the dwarfs should be homozygous double mutants and should yield, assuming an additive effect of the two mutations, seedlings with a higher reduction in their length than single gene mutants. This is exactly what we observed and can be taken as evidence that the two genes have an additive effect (Table 1, Figure 1).

Table 1. Stature of wild type, dwarf and severe dwarf seedlings and frequency of the severe dwarf phenotype in the selfed progeny of different double heterozygous combinations. The *d*10/an1* double mutant is included as another example of the detection of severe dwarfs in the F2.

Double heterozygote constitution (F2)	Seedling elongation (cm)			Frequency of severe dwarfs (%)		P-value
	wild type	dwarfs	severe dwarfs	observed	expected	
*d*11* x *d2*	18.2	7.2	4.4	18.7	14.3	0.15
*d*11* x *an1*	22.9	9.4	6.1	15.6	14.3	0.56
*d*10* x *an1*	16.6	8.9	4.5	17.4	14.3	0.38