Amazing grass: developmental genetics of maize domestication

E. Vollbrecht[1] and B. Sigmon
Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, U.S.A.

Abstract

Crop plants were domesticated by prehistoric farmers through artificial selection to provide a means of feeding the human population. This article discusses the developmental genetics of crop domestication and improvement, including the historical framework and recent approaches in maize and other grasses. In many cases, selecting for a plant form that correlates with productivity involves controlling meristem activity. In the domestication of modern maize from its progenitor *Zea mays* ssp. *parviglumis*, QTL (quantitative trait loci) mapping, genetics and population genomics approaches have identified several genes that contain signatures of selection. Only a few genes involved in the derivation of the highly productive maize ear have been identified, including *teosinte glume architecture1* and *ramosa1*. Future prospects hinge on forward and reverse genetics, as well as on other approaches from the developing discipline of evo-devo (evolutionary developmental biology).

Introduction

Plants, through their ability to harvest light energy and convert it into chemical energy, are the primary producers on which life on earth depends. Not surprisingly then, humans have domesticated plants by cultivation and selection, ultimately modifying them for particular traits [1]. Most notably, the grasses, including grain crops, are sources of commodities that, either directly or indirectly, literally feed the human world. Prehistoric farmers domesticated grasses by selecting plants with patterns of growth and development that enhanced food production. By doing so, they placed strong artificial selection on particular alleles of genes, and on gene combinations, involved in these developmental processes. While it is expected that at least hundreds of genes were selected in the domestication of a typical crop plant, until very recently, the identity of these genes was almost completely unknown. In this article, we discuss the history and developmental genetics of crop domestication with a focus on recent approaches in the grasses and work that bears on the remarkable maize ear.

The plant body is produced by the activity of specialized multicellular tissues called meristems. Meristems are formed during embryogenesis and persist during plant ontogeny as perpetually embryonic tissues, generating new organs at the growing tips of roots (root apical meristem or RAM) and shoots (shoot apical meristem or SAM) [2]. In the shoot, the source of grass commodities, the SAM produces lateral organs such as leaves and flower parts, produces the stems that bear organs, and renews itself to continue functioning [3] (Figure 1). Hence the construction of a plant shoot, or plant shoot architecture, is dictated largely by the activity of SAMs. Relevant parameters of SAM activity include the duration of activity which determines stem or branch length, the pattern of organ and branch initiation, the orientation of branches in space and the types of organs initiated, all of which contribute to the characteristic forms of familiar vegetative trees, shrubs and, of course, grasses [4]. Thus, when a shoot produces a branch system that bears flowers, known as an inflorescence, a collection of meristems must be coordinately regulated (Figure 1). The inflorescence, as a specialized type of shoot, has its architecture governed by the same basic parameters of meristem activity. In summary, the architecture of whole plants or of major plant regions, such as the inflorescence, is dictated largely by the activity of meristems, so that changes in architecture may often be associated with changes in regulation of meristem activity.

Plant domestication and crop improvement

During the agricultural revolution that began roughly 10 000 years ago, humans started cultivating animals and crop plants as food sources. Continued crop improvement by relatively rapid modern breeding methods means that crop derivation may be considered to be a two-step process, consisting of domestication followed by improvement. Among the most notable improvements in grass crops, the so-called green revolution of the late 20th century, was breeding for modified plant architecture. Improved rice and wheat varieties with short stature and spectacular yield increases were developed independently, yet the same endogenous plant hormone (gibberellin) pathway was altered in both cases [1]. Thus developmental mechanisms in common between rice and wheat make it possible to generalize the beneficial effect of altering the same gene activity in different species.

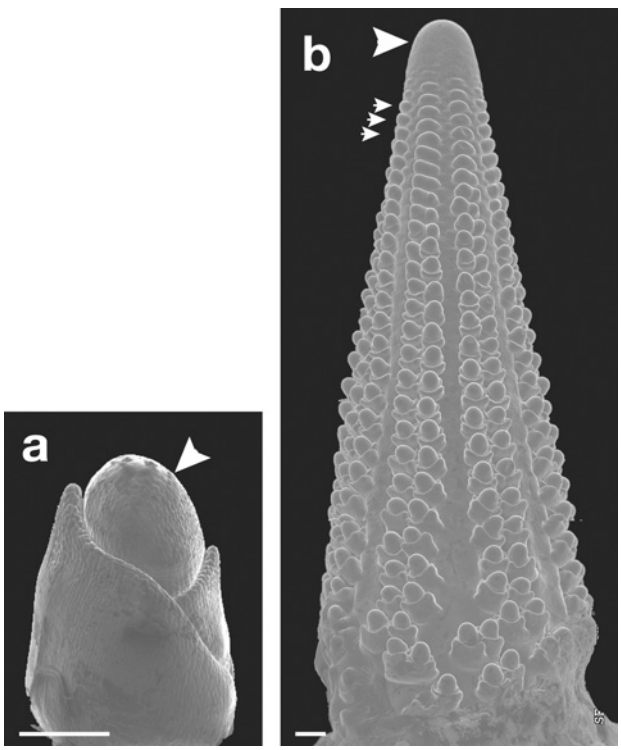
Key words: developmental genetics, meristem, plant architecture, plant domestication, *ramosa1*, transcription factor.

Abbreviations used: *ba1*, *barren stalk1*; QTL, quantitative trait loci; *ra1*, *ramosa1*; SAM, shoot apical meristem; *tb1*, *teosinte branched1*; *tga1*, *teosinte glume architecture1*.

[1]To whom correspondence should be addressed (email vollbrec@iastate.edu).

©2005 Biochemical Society

Figure 1 | **Maize shoot apical meristems**
(**a**) Dome of the vegetative meristem (arrowhead) flanked by leaf primordia. (**b**) Inflorescence primordium consists of a large terminal meristem (large arrowhead) and abundant lower-order meristem domes (a few are indicated by small arrowheads). Scale bar, 100 μm.



On the other hand, a similar trait in improved sorghum results from disrupted transport of a different hormone, auxin [5], although interfering with gibberellins does induce dwarfism in sorghum. This suggests that, because sorghum's hormone physiology relates differently to plant stature, the auxin pathway is a more useful agricultural target. A related example involves shoot architecture in rice and maize [6]. Both plants are capable of producing tillers, side shoots that recapitulate the main shoot. In rice, increased tillering leads to increased panicle production and therefore more grain [7]. In maize, however, increased tillering leads to excessive vegetative growth and thus less grain. In fact, in maize, there has been strong selection for the opposite phenotype, decreased tillering [8,9]. These examples from crop improvement underline that, owing to physiological and developmental similarities and differences between crops, as we unravel myriad changes that are involved in plant domestication, we can expect to see similarities and differences in the underlying mechanisms of particular changes.

Similar to crop improvement, crop domestication involves a relatively restricted initial subpopulation and includes strong selection, so that a population bottleneck leads to reduced genetic diversity of the crop relative to its ancestor [10,11]. Reduced genetic diversity due to bottlenecks has important implications for breeding and further improvement [10], and is manifest as reduced nucleotide diversity across the genome. Notably, a gene that is responsible for a trait selected during domestication will experience an extreme bottleneck that removes most or even all genetic variation from the target gene(s) [11]. Loci that were the targets of such selective sweeps can therefore be identified, because, in extant plants, they bear so-called signatures of selection: their nucleotide diversity may be even lower than that of typical neutral genes. This provides a method to identify selected genes through genome-wide scans of patterns of nucleotide diversity. This fact has been exploited in a number of genomics-scale approaches that are revealing how genomes are shaped by domestication and evolution [12]. For example, the recent rapid progress in maize genome sequencing [13] has enabled genome-scan studies of nucleotide diversity [14,15], which indicate that roughly 3% of maize genes contain signatures of selection. If the samples in these studies are representative, approx. 1200 genes were targeted during maize domestication.

Maize domestication

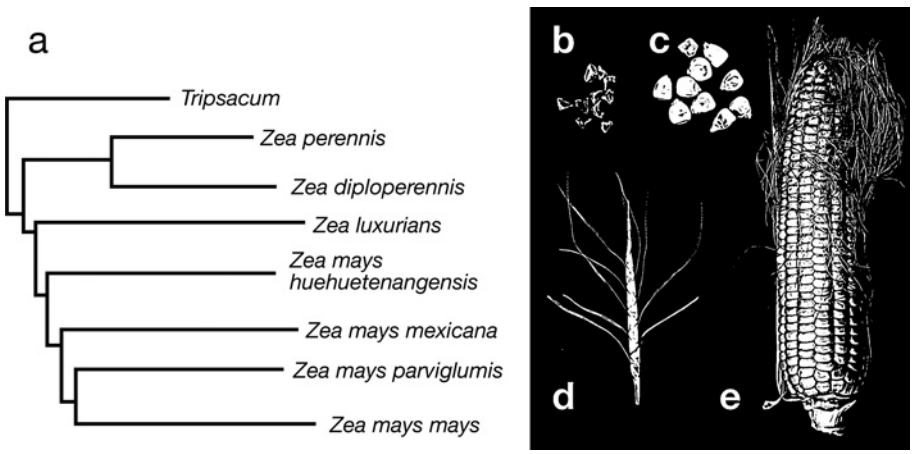
Archaeological evidence from ancient corncobs indicates that maize domestication occurred between 5000 and 10 000 years ago [16]. Recent molecular evidence corroborates these data. Beginning roughly 9000 years ago in the Balsas River Valley in Southern Mexico, modern domestic maize was derived from *Zea mays* ssp. *parviglumis*, known as a teosinte [17]. Teosinte, derived from 'teocintli' from the Nahuátl Indians for 'grain of the gods', refers to a group of annual (*Zea luxurians* and *Zea mays*) and perennial (*Zea perennis* and *Zea diploperennis*) species of the genus *Zea*, which is indigenous to Mexico and Central America [18,19] (Figure 2a). Teosintes and maize have similar growth forms, but with such major differences in plant architecture that taxonomists had once placed each in a separate genus [20]. The female inflorescence, known as the ear, presents some of these stunning differences (Figure 2). The teosinte ear produces only 5–12 kernels, enclosed in hardened fruit cases that disperse as the ear disarticulates. The modern maize ear has several hundred kernels, each firmly attached to the cob and lacking the protection of a stony casing. Therefore maize, as a cultivated plant, depends on human aid for survival, because the kernels are easily consumed and digested by animals, and there is no intrinsic mechanism for dispersion. Maize and annual teosintes are cytologically similar and are interfertile, begging the question of the basis of such extreme morphological differences.

A solution to this conundrum was proposed by George Beadle in the form of the teosinte hypothesis, which portrayed teosinte as the single progenitor of maize [21]. Beadle suggested that ancient peoples cultivated teosinte as a source of food and that, during cultivation, mutations arose that improved its usefulness and were therefore selected upon. Beadle also hypothesized that as few as five major mutations would be sufficient to convert teosinte into a primitive form of domestic maize, and that humans selected other major and minor mutations over time. Beadle's experimental

©2005 Biochemical Society

Figure 2 | **Teosintes and maize**

(**a**) Phylogeny with sister species *Tripsacum* as outgroup. Kernels (**b** and **c**) and ripening ears (**d** and **e**) of teosinte and modern maize respectively.



evidence included crosses between maize and teosintes, in which he correlated interfertility with normal chromosome pairing [22] and, working with Emerson, established a similar frequency of cross-over events in maize–maize and maize–teosinte hybrids [23]. Based on such genetic evidence, Beadle concluded that maize was a domesticated form of teosinte [24].

Molecular evidence has supported many of Beadle's ideas. A study to locate QTL (quantitative trait loci) involved in morphological differences between maize and teosinte found five QTL of strong effect [25]. Two or three of these QTL have recently been resolved to probable individual genes, all of which encode transcriptional regulators [8,29,31]. Moreover, each maize gene bears a strong signature of selection. The first such gene identified was *tb1* (*teosinte branched1*), so named because *tb1* mutants have a plethora of tillers tipped with male inflorescences, like teosinte [26,27]. *tb1* controls the fate of axillary meristems by repressing organ growth in tissues where it is expressed; maize alleles express *tb1* mRNA at higher levels than teosinte alleles [8]. These data suggest that, in selecting a plant architecture trait, an RNA expression difference between maize and teosinte was selected for, which is consistent with the finding of a signature of selection that is confined to the *tb1* upstream promoter region [28]. A selected gene that maps to a second of the five regions, *ba1* (*barren stalk1*), also affects plant architecture. *ba1* is required for the initiation of all aerial axillary meristems, leading to the speculation that *ba1* may interact with *tb1* to regulate vegetative shoot architecture [29]. Finally, the *tga1* (*teosinte glume architecture1*) gene probably corresponds to a third QTL. Maize forms of *tga1* confer soft glumes, while teosinte forms confer a hardened stony fruit case [30]. *tga1* was recently cloned and the selected maize *tga1* alleles appear to be mutant relative to extant teosinte [31]. This is unlike *tb1*, *ba1* and most selected genes that have been identified where the selected alleles are also found in extant teosinte at moderate frequency. Thus, while many elements of the teosinte hypothesis hold up, it is notable that the spectacular morphological difference between maize and teosinte may be substantially attributed not to mutation as Beadle hypothesized, but simply to novel combinations of the tremendous genetic diversity of teosinte [11]. These studies also support the hypothesis that if selection acts on transcription factors and/or their *cis*-regulatory regions, then small genetic changes may have a profound impact on morphology [32].

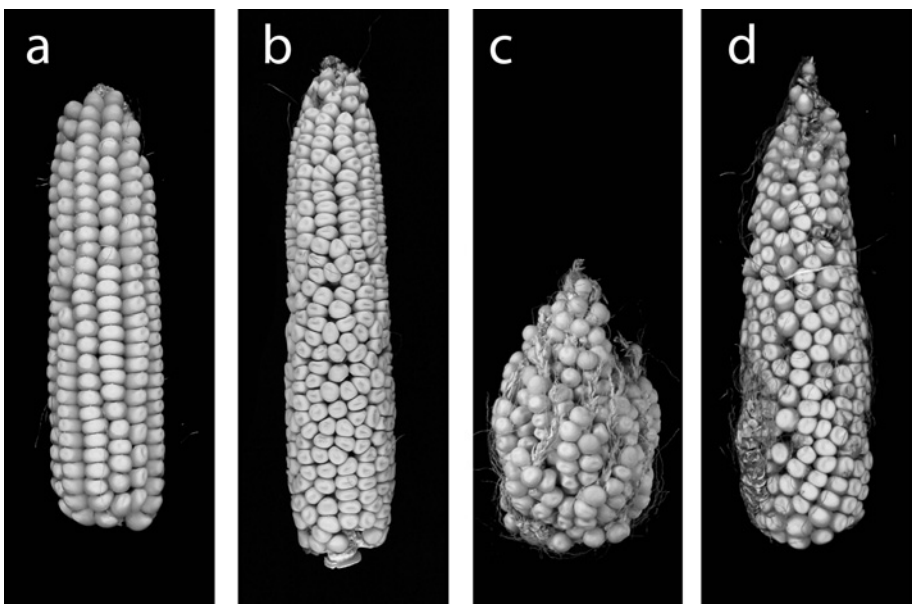
The maize inflorescence

While the predominant target when Native Americans domesticated maize was productivity in the ear, the derivation of ear morphology is not well explained by the five major QTL and has remained a mystery. Forward-genetic analysis of inflorescence architecture may be a useful route to identifying these genes of minor effect that were targets of selection, when the efforts focus on genes that affect inflorescence characters known or predicted to have been under selection (Maize Inflorescence Project: http://www.maizegdb.org/mip/). Work over the last few years with maize mutants exemplifies this approach. Maize's rich history as a genetic research organism includes classical inflorescence mutants that were identified and preserved by researchers from the turn and the early part of the 20th century [34–41]. At this time, some mutants were considered separate species or revertant evolutionary throwbacks because of morphological similarities to undomesticated grasses, and common ancestry of maize and teosinte, based on comparative morphology, was already favoured, although not without dissent [42,43]. By the mid-century, many single gene mutants were known [44]; by the end of the century, mutants affecting many aspects of inflorescence development had been initially characterized [45], and the work of Doebley and Stec [25,46] had

©2005 Biochemical Society

Figure 3 | **Maize ears at maturity**

(**a**) Normal. (**b**) Mutant homozygous *ra1-RS* weak allele. Slightly reduced gene activity leads to crooked rows. (**c**) Mutant homozygous *ra1-R* strong allele, with long branches. (**d**) Mutant homozygous *ra2-R* has crooked rows and some branching.



affirmed that the genetics of ear domestication were not simply explained by QTL. The inflorescence mutants have represented a relatively untapped resource for elucidating relationships between developmental molecular genetics and domestication.

Among the classical inflorescence mutants that have been preserved and whose spontaneous origin is clearly documented, *ra1* (*ramosa1*) is perhaps the oldest [35]. Almost a century later, molecular analysis [47] reveals that *ra1* encodes a transcription factor that regulates meristems to control the branching architecture of inflorescences (Figure 3). At least one other classical mutant, *ramosa2*, controls branching similarly, through the *ra1* genetic pathway. *ra1* acts by establishing a boundary between lower-order meristems and the principal inflorescence axis, thereby controlling the fundamental property of the duration of meristem activity. Reduced nucleotide diversity at *ra1* in modern maize implies that the gene was a target of selection during domestication or improvement. While the original loss-of-function mutant conferred extreme ear branching, intermediate levels of *ra1* gene activity lead to ears with crooked rows (Figure 3), suggesting selection for *ra1* forms that preserved straight rows in the massive ear of domesticated maize. For other cloned branching genes, including another gene whose activity is required for straight rows, evidence for selection has not been reported [48,49]. Darwin considered selection under domestication as a model for selection under evolution, an idea examined recently with respect to maize *tga1* [31]. Corollaries of this idea are that genes selected for during domestication are candidates for genes selected for during evolution, and vice versa. In the context of inflorescence branching architecture, the presence or absence of long branches varies across the grasses. Indeed, *ra1* is absent in long-branched rice, and shows differential regulation in other grasses that is also consistent with this hypothesis [47]. Given its role in modulating long-branch architecture, it will be interesting to determine the origin of *ra1* within the grasses.

Prospects

Enquiry into the domestication of development closely parallels that into plant evo-devo (evolutionary developmental biology) [50], and substantial advances in both arenas appear imminent. Among the grasses whose inflorescences produce grain crops and have accordingly been subjected to intense selective pressure during domestication, the molecular genetics of inflorescence development are best understood in maize and rice [51,52], so resources in these plants will figure prominently. While a profusion of putative domestication genes will come from genome scans, it will be more difficult to unravel the plant traits that were selected for and are associated with these genes, in part because the associated phenotypic diversity has been eliminated from the experimental organism. Candidate loci must be verified by characterizing their phenotypic effects, an effort that requires functional analysis of individual genes. Fortunately, burgeoning EST (expressed sequence tag), microarray and functional genomics resources are available in maize ([53,54], and Ac/Ds functional genomics in maize, http://www.plantgdb.org/prj/AcDsTagging/, and TIGR Gene Indices, http://www.tigr.org/tdb/tgi/plant.shtml). Complementary to genomics, forward-genetic approaches that might identify ear domestication genes include QTL experiments, such as those involving multiple teosinte–maize populations (Maize

©2005 Biochemical Society

Diversity Project: http://www.panzea.org/), and forward-genetic analysis of inflorescence architecture, which should continue to inform directly and through identification of gene networks (Maize Inflorescence Project: http://www.maizegdb.org/mip/). Ultimately, examination of wild alleles in the domesticated species should also prove highly informative, by transgenic means or as shown for teosinte alleles in maize [28,31].

References

1 Khush, G.S. (2001) Nat. Rev. Genet. **2**, 815–822
2 Steeves, T.A. and Sussex, I.M. (1989) Patterns in Plant Development, 2nd edn., Cambridge University Press, Cambridge
3 Carles, C.C. and Fletcher, J.C. (2003) Trends Plant Sci. **8**, 394–401
4 Sussex, I.M. and Kerk, N.M. (2001) Curr. Opin. Plant Biol. **4**, 33–37
5 Multani, D.S., Briggs, S.P., Chamberlin, M.A., Blakeslee, J.J., Murphy, A.S. and Johal, G.S. (2003) Science **302**, 81–84
6 Sawers, R.J., Sheehan, M.J. and Brutnell, T.P. (2005) Trends Plant Sci. **10**, 138–143
7 Li, X., Qian, Q., Fu, Z., Wang, Y., Xiong, G., Zeng, D., Wang, X., Liu, X., Teng, S., Hiroshi, F. et al. (2003) Nature (London) **422**, 618–621
8 Doebley, J., Stec, A. and Hubbard, L. (1997) Nature (London) **386**, 485–488
9 Sprague, G.F. and Dudley, J.W. (eds.) (1988) Corn and Corn Improvement, 3rd edn., American Society of Agronomy, Crop Science Society of America and Soil Science Society of America, Madison
10 Tanksley, S.D. and McCouch, S.R. (1997) Science **277**, 1063–1066
11 Buckler, IV, E.S., Thornsberry, J.M. and Kresovich, S. (2001) Genet. Res. **77**, 213–218
12 Buckler, IV, E.S. and Thornsberry, J.M. (2002) Curr. Opin. Plant Biol. **5**, 107–111
13 Messing, J., Bharti, A.K., Karlowski, W.M., Gundlach, H., Kim, H.R., Yu, Y., Wei, F., Fuks, G., Soderlund, C.A., Mayer, K.F. and Wing, R.A. (2004) Proc. Natl. Acad. Sci. U.S.A. **101**, 14349–14354
14 Wright, S.I., Bi, I.V., Schroeder, S.G., Yamasaki, M., Doebley, J.F., McMullen, M.D. and Gaut, B.S. (2005) Science **308**, 1310–1314
15 Vigouroux, Y., Jaqueth, J.S., Matsuoka, Y., Smith, O.S., Beavis, W.D., Smith, J.S. and Doebley, J. (2002) Mol. Biol. Evol. **19**, 1251–1260
16 Smith, B. (1995) The Emergence of Agriculture, Freeman, New York
17 Doebley, J. (2004) Annu. Rev. Genet. **38**, 37–59
18 Doebley, J. (1990) Econ. Bot. **44** (suppl. 3), 6–27
19 Fukunaga, K., Hill, J., Vigouroux, Y., Matsuoka, Y., Sanchez, G.J., Liu, K., Buckler, IV, E.S. and Doebley, J. (2005) Genetics **169**, 2241–2254
20 Wilkes, H.G. (1967) Teosinte: the Closest Relative of Maize, Bussey Institute, Harvard University, Cambridge
21 Beadle, G. (1939) J. Hered. **30**, 245–247
22 Beadle, G. (1932) Z. Indukt. Abstamm. Vererbungsl. **62**, 291–304
23 Emerson, R. and Beadle, G.W. (1932) Z. Indukt. Abstamm. Vererbungsl. **62**, 305–315
24 Beadle, G. (1972) Field Mus. Nat. Hist. Bull. **43**, 2–11
25 Doebley, J. and Stec, A. (1993) Genetics **134**, 559–570
26 Burnham, C.R. (1959) Maize Genet. Coop. News Lett. **33**, 74
27 Doebley, J., Stec, A. and Gustus, C. (1995) Genetics **141**, 333–346
28 Wang, R.L., Stec, A., Hey, J., Lukens, L. and Doebley, J. (1999) Nature (London) **398**, 236–239
29 Gallavotti, A., Zhao, Q., Kyozuka, J., Meeley, R.B., Ritter, M.K., Doebley, J.F., Pe, M.E. and Schmidt, R.J. (2004) Nature (London) **432**, 630–635
30 Dorweiler, J.E. and Doebley, J. (1997) Am. J. Bot. **84**, 1313–1322
31 Wang, H., Nussbaum-Wagler, T., Li, B., Zhao, Q., Vigouroux, Y., Faller, M., Bomblies, K., Lukens, L. and Doebley, J.F. (2005) Nature (London) **436**, 714–719
32 Doebley, J. and Lukens, L. (1998) Plant Cell **10**, 1075–1082
33 Reference deleted
34 Sturtevant, E. (1894) Bull. Torrey Bot. Club **21**, 319–343
35 Gernert, W. (1912) Am. Nat. **46**, 616–622
36 Collins, G. (1917) J. Agric. Res. **9**, 383–413
37 Hayes, H.K. and Brewbaker, H.E. (1928) J. Hered. **19**, 561–567
38 Kempton, J. (1921) U.S. Dep. Agric. Bull. **971**, 1–20
39 Kempton, J.H. (1923) J. Hered. **14**, 243–252
40 Kempton, J.H. (1934) J. Hered. **25**, 29–32
41 Phipps, I.F. (1928) J. Hered. **19**, 399–404
42 Weatherwax, P. (1918) Bull. Torrey Bot. Club **45**, 309–342
43 Weatherwax, P. (1919) Bull. Torrey Bot. Club **46**, 275–278
44 Nickerson, N.H. and Dale, E.E. (1955) Ann. Mo. Bot. Gard. **42**, 195–211
45 Veit, B., Schmidt, R.J., Hake, S. and Yanofsky, M.F. (1993) Plant Cell **5**, 1205–1215
46 Doebley, J. and Stec, A. (1991) Genetics **129**, 285–295
47 Vollbrecht, E., Springer, P.S., Goh, L., Buckler, IV, E.S. and Martienssen, R. (2005) Nature (London) **436**, 1119–1126
48 Chuck, G., Muszynski, M., Kellogg, E., Hake, S. and Schmidt, R.J. (2002) Science **298**, 1238–1241
49 Chuck, G., Meeley, R. and Hake, S. (1998) Genes Dev. **12**, 1145–1154
50 Kellogg, E.A. (2004) Curr. Opin. Plant Biol. **7**, 92–98
51 McSteen, P., Laudencia-Chingcuanco, D. and Colasanti, J. (2000) Trends Plant Sci. **5**, 61–66
52 Bommert, P., Satoh-Nagasawa, N., Jackson, D. and Hirano, H.Y. (2005) Plant Cell Physiol. **46**, 69–78
53 May, B.P., Liu, H., Vollbrecht, E., Senior, L., Rabinowicz, P.D., Roh, D., Pan, X., Stein, L., Freeling, M., Alexander, D. and Martienssen, R. (2003) Proc. Natl. Acad. Sci. U.S.A. **100**, 11541–11546
54 Henikoff, S., Till, B.J. and Comai, L. (2004) Plant Physiol. **135**, 630–636

Received 16 August 2005

©2005 Biochemical Society