Segregations for Onion Bulb Colors Reveal That Red Is Controlled by at Least Three Loci

Anil Khar

Department of Horticulture, University of Wisconsin, Madison, WI 53706 USA; and
National Research Centre for Onion and Garlic, Rajgurunagar, Pune Dist., Maharashtra,
India, 410505

Jernej Jakse

Department of Horticulture, University of Wisconsin, Madison, WI 53706 USA; and Biotechnical
Faculty, University of Ljubljana, Jamnikarjeva 101, Ljubljana 1000, Slovenia

Michael J. Havey¹

USDA-ARS, Department of Horticulture, 1575 Linden Drive, University of Wisconsin, Madison,
WI 53706 USA

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ABSTRACT. Onion (*Allium cepa* L.) bulb color is controlled by at least five major loci (*I*, *C*, *G*, *L*, and *R*) and seedcoat color by one locus (*B*). The authors developed families segregating for bulb and seedcoat colors, simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs) in genomic amplicons of dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANS). The *B* and *C* loci were linked to SSRs on chromosomes 1 and 6 respectively. For all of three families, SNPs in DFR cosegregated with the *R* locus conditioning red bulb color. In the family from B2246 × B11159, red bulbs versus yellow bulbs were controlled by DFR and a locus (*L2*) linked at 6.3 cM to ANS. The authors propose that yellow bulb onions have been independently selected numerous times and that yellow populations carry independent mutations in structural or regulatory genes controlling the production of red bulb color in onion.

Bulb color in onion is an economically important trait and is conditioned by the interaction of at least five major loci (Clarke et al., 1944; El-Shafie and Davis, 1967; Reiman, 1931). White bulbs result from a dominant allele at the *I* locus or recessive alleles at the *C* locus. Colored bulbs (chartreuse, light red, red, or yellow) require the homozygous recessive genotype at the *I* locus and a dominant allele at the *C* locus. In plants that are *iiC-*, a dominant allele at the *G* locus produces golden yellow bulbs, and the homozygous recessive genotype (*iiC-gg*) conditions chartreuse bulbs. Light-red bulbs are produced when the plant is *iiC-G-* and has dominant alleles at both the *L* and *R* loci; bulbs with deeper red colors are produced when both the *L* and *R* loci are homozygous dominant (El-Shafie and Davis, 1967). If either the *L* or *R* locus is homozygous recessive, yellow bulbs are produced. El-Shafie and Davis (1967) proposed that light-red bulb color produced when crossing two yellow onions [termed complementary light red by Jones and Peterson (1952)] results when the yellow parental plants have the genotypes *iiCCGGLlrr* and *iiCCGGllRR*.

Onion bulbs accumulate significant amounts of flavonoids, such as quercetin derivatives in yellow and anthocyanins in red

bulbs (Fossen et al., 1996; Tsushida and Suzuki, 1995). Many of the structural genes in the flavonoid biosynthetic pathway have been cloned (Holton and Cornish, 1995). Kim et al. (2004a, 2005c) correlated expression and segregation of dihydroflavonol 4-reductase (DFR) with yellow versus red bulb colors. In a confusing series of papers, variation at anthocyanidin synthase (ANS) was associated first with a new recessive locus (*p*) conditioning pink bulbs (Kim et al., 2004b, 2005b), and then subsequently with four alleles at the *L* locus [*ANS-p* for recessive pink, *ANS-l* and *ANS-L* for the Brazilian and North American alleles of the complementary light-red system respectively (Kim et al., 2005a), and *ANS-h1* for dark red (Kim et al., 2006)]. No candidate genes have been identified for the *I*, *C*, or *G* loci.

In this study we developed families segregating for dominant white (*I-*), recessive white (*cc*), yellow (*iiC-llR-*, *iiC-L-rr*, and *iiC-llrr*), and light-red to red (*iiC-L-R-*) bulb colors, as well as black (*B-*) versus brown (*bb*) seeds (Davis, 1966). We scored segregations for simple sequence repeats (SSRs), bulb and seed colors, and single nucleotide polymorphisms (SNPs) in ANS and DFR to assess linkages among these important morphological and molecular markers. Our results support DFR as the *R* locus and reveal a new locus (*L2*) linked at 6.3 cM to ANS that conditions red bulb color in onion.

Materials and Methods

We obtained from N. Molenaar (formerly of Crookham Seed Co., Caldwell, ID) seed of a white onion (B11159) that produced flattened bulbs when grown in southern Wisconsin. Randomly selected bulbs from B11159 were individually

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Names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

¹Corresponding author. E-mail: mjhavey@wisc.edu.

self-pollinated to produce S_1 families and testcrossed to MSU5718A $\times$ MSU8155B, a yellow bulb, male-sterile hybrid. Randomly selected B11159 plants were individually crossed as the male with plants from the inbred B2246, which has yellow bulbs (*iiCCL-rr* or *iiCCLrr*) and brown (*bb*) seeds. All self-pollinations and crosses were completed with mesh cages and house flies [*Musca domestica* L. (Rincon-Vitova Insectaries, Ventura, CA)] as described by Havey (1993). Hybrids were identified by white bulb color for progenies from B2246 as the maternal parent. Individual F_2 plants were self-pollinated and testcrossed to the yellow bulb (*iiCCL-rr* or *iiCCLrr*), black-seed (*BB*), male-sterile (*S msm*) hybrids MSU5718A $\times$ MSU8155B, B3350A $\times$ B2352B, or B1750A $\times$ B1794B. At least 50 F_3 and testcross plants were scored for white or beige, red or light red, versus yellow bulb colors in field plots over at least 3 years. No effort was made to distinguish white from beige or red from light red. F_3 seed was scored for black versus brown colors to assign genotypes at the *B* locus to F_2 plants. Goodness-of-fit to expect ratios for F_2 and testcross families were completed using chi-square analyses on pooled data after confirming homogeneity of ratios (Gomez and Gomez, 1984).

A second family segregating for red versus yellow bulbs was developed from an inbred population (B8667) derived from a cross of U.S. Department of Agriculture Plant Introduction (PI) 262985 with the red inbred line B5361. Random bulbs from the F_1 MSM $_3$ generation (where M is mass random pollinations among three to five bulbs) were self-pollinated and one family was segregated for yellow versus red bulbs. Random plants from this F_1 MSM $_3$ S generation were again self-pollinated to produce the F_1 MSM $_3$ S $_2$ family and numbers of red bulbs versus yellow bulbs were scored over at least 3 years.

The third segregating family was BYG15-23 $\times$ AC43. We previously reported segregation of complementary light-red bulb color in testcrosses of F_2 plants from this family with male-sterile lines (King et al., 1998).

Polymorphisms in ANS and DFR have been associated with red versus yellow bulb colors in onion (Kim et al., 2004b,

2005b, c, 2006). Amplicons from ANS and DFR were generated using primers across promoter (Kim et al., 2004a, b, 2005b, 2005c, 2006) and coding regions (5'-CGTGATAATTGCAC CACCAG and 5'-ACAACCTCCGTTCCAGATGC for ANS and 5'-ATCAGCGGCTCTATTGCTGT and 5'-TCCCCA CATGCTCAATTAAA for DFR). Polymerase chain reactions (PCRs) had 50 ng onion genomic DNA, 1 μ M each primer, 250 μ M deoxyribonucleotides (dNTPs), 5 U Taq Polymerase (Promega, Madison, WI), 1.5 mM MgCl $_2$, 50 mM KCl, 10 mM Tris-HCl (pH, 9.0), and 0.1% Triton X-100 (Sigma, St. Louis, MO) in a total volume of 25 μ L. Cycling conditions were 94 $^{\circ}$ C for 5 min. followed by 39 cycles of 92 $^{\circ}$ C for 1.15 min., 58.7 (DFR) or 60.6 (ANS) $^{\circ}$ C for 2 min., and 72 $^{\circ}$ C for 2 min. Amplicons were gel purified and sequenced to confirm identity with ANS or DFR. DNA from parents and progenies of the three segregating families were scored for SNPs in the ANS and DFR coding regions.

Linkages were assessed among the major bulb and seed color loci, ANS, and DFR, and SSR markers. Procedures for DNA isolations from families, primer sequences, and production of genomic amplicons carrying SSRs were described by Martin et al. (2005) and Jakse et al. (2005). Simple sequence repeats segregating in the B2246-by-B11159 family were ACM006, ACM013, ACM045, ACM054 (5'-AAAGATGGTT TGTTGGTGGC and 5'-GAGTGAGAGGGGAAATGGAA), ACM066, ACM068 (5'-CGAAGGTGAAGGTGTACGGT and 5'-CAAATGGCTGCAATAAGCAA), ACM071, ACM093, ACM101, ACM102, ACM119, ACM124, ACM132, ACM138, ACM146, ACM151, ACM152, ACM154, ACM177, ACM218 (5'-CAACGGGCTATTTCCG TAGA and 5'-AATGCATGCTTTTGACCAC), ACM221, and ACM251. In the B8667 family, segregating SSRs were ACM006, ACM013, ACM045, ACM119, ACM132, ACM146, ACM151, ACM180, and ACM221. Many of these SSRs have been placed on the BYG15-23 $\times$ AC43 genetic map (Martin et al., 2005). MapManager (ver. 0.30, Center of Excellence in Bioinformatics and Life Sciences, Buffalo, NY) (Manly et al., 2001) was used at $P < 0.001$ with the Kosambi

Table 1. Segregations of onion bulb colors in S_1 , F_2 , and testcross families from B2246 by B11159.

Pedigree ^z	Gen. ^y	Fam. ^x	Obs. ^w			Exp. ^w			Assigned genotypes	<i>P</i> value ^v
			W	R	Y	W	R	Y		
A $\times$ B11159	TC	4	348	0	0	1	0	0		
B11159 $\otimes$	S1	4	182	0	0	1	0	0	<i>II</i>	
A $\times$ (B2246 $\times$ B11159)	TC	6	232	103	92	2	1	1		0.152
(B2246 $\times$ B11159) $\otimes$	F2	6	351	60	21	208	22	21	<i>liCcLlRr</i>	0.005
A $\times$ (B2246 $\times$ B11159)	TC	2	112	76	57	2	1	1		0.093
(B2246 $\times$ B11159) $\otimes$	F2	2	111	12	15	208	27	21	<i>liCcLlRr</i>	0.437
A $\times$ (B2246 $\times$ B11159)	TC	1	0	49	33	2	1	1		0.210
(B2246 $\times$ B11159) $\otimes$	F2	1	6	10	4	16	27	21	<i>iiCcLlRr</i>	0.837
A $\times$ (B2246 $\times$ B11159)	TC	2	122	0	81	1		1		0.004
(B2246 $\times$ B11159) $\otimes$	F2	2	132	0	23	13		3	<i>liCcLlrr</i>	0.212
A $\times$ (B2246 $\times$ B11159)	TC	1	47	16	23	2	1	1		0.390
(B2246 $\times$ B11159) $\otimes$	F2	1	55	22	3	28	12	2	<i>liCcLLRr</i>	0.106

^zA, yellow bulb, male-sterile plant with expected genotype *iiCCLLrr*.

^yGen., generation; TC, testcross.

^xNumbers of families (Fam.).

^wObserved (Obs.) and expected (Exp.) ratios of white (W), red (R), and yellow (Y) bulbs. Data were pooled across families after establishing homogeneity of ratios.

^vProbability (*P*) of goodness-of-fit from chi-square analyses.

mapping function to assess linkages among SNPs in ANS and DFR, SSRs, and the bulb and seed color loci.

Copy numbers of ANS and DFR in the onion genome were estimated by hybridization of PCR amplicons to DNAs isolated from leaf tissue combined from at least 25 F₃ progenies from the B2246 × B11159 family. Digestion of DNAs with *EcoRI*, *EcoRV*, or *HindIII*, gel electrophoresis, labeling and hybridization conditions, and autoradiography were described by King et al. (1998).

Results and Discussion

SEGREGATIONS FOR BULB AND SEED COLOR LOCI IN THE B2246 × B11159 FAMILY. All seed from self-pollination of random bulbs from B11159 was black (*BB*) and produced only white bulbs (Table 1). In crosses of B11159 plants to yellow bulb, male-sterile lines, only white bulbs were produced (Table 1), revealing that B11159 was homozygous dominant at the *I*

locus. The testcross and F₂ progenies from hybrids between B2246 by B11159 segregated for white, red, and yellow bulbs (Table 1). No chartreuse bulbs were produced.

One family (17400) from B2246 × B11159 was chosen for further study because it segregated for bulb and seed colors. Individual F₂ plants were self-pollinated to produce F₃ families and crossed to male-sterile plants to produce the testcross generations. Because these male-sterile lines produce only yellow bulbs in crosses with North American long-day, yellow bulb inbreds, they should be homozygous recessive at the *R* locus; however, they could carry dominant alleles at the *L* locus (El-Shafie and Davis, 1967; Jones and Peterson, 1952). Testcross segregations for bulb colors fit expected ratios for 46 of 51 families, assuming that the yellow bulb, male-sterile lines were *iiCCLLrr* or less commonly *iiCCLlrr* (Table 2). F₃ segregations fit expected ratios for 45 of 55 F₃ families, excluding 30 families with all-white bulbs (*II* or *cc*). Segregations revealed that B2246 must have contributed

Table 2. Segregations of onion bulb colors in F₃ and testcross families from B2246 × B11159.

Pedigree ^z	Gen. ^y	Assigned genotype	Fam. ^x	Obs. ^w			Exp. ^w			<i>P</i> value ^v
				W	R	Y	W	R	Y	
A × [(B2246 × B11159) ⊗]	TC		27	1721	0	0	1	0	0	
[(B2246 × B11159) ⊗] ⊗	F3	<i>II</i> —	27	883	0	0	1	0	0	
A × [(B2246 × B11159) ⊗]	TC		3	112	131	0	1	1	0	0.476
[(B2246 × B11159) ⊗] ⊗	F3	<i>liCCLLRr</i>	3	102	20	0	3	1	0	0.090
A × [(B2246 × B11159) ⊗]	TC		2	60	36	33	2	1	1	0.681
[(B2246 × B11159) ⊗] ⊗	F3	<i>liCCLLRr</i>	3	111	34	18	12	3	1	0.025
A × [(B2246 × B11159) ⊗]	TC		10	378	219	191	2	1	1	0.193
[(B2246 × B11159) ⊗] ⊗	F3	<i>liCCLlRr</i>	12	494	101	68	48	9	7	0.622
A × [(B2246 × B11159) ⊗]	TC		1	30	15	14	2	1	1	0.975
[(B2246 × B11159) ⊗] ⊗	F3	<i>liCCllRr</i>	1	21	9	4	48	9	7	0.103
A × [(B2246 × B11159) ⊗]	TC		2	58	36	0	1	1	0	0.769
[(B2246 × B11159) ⊗] ⊗	F3	<i>liCcLLRR</i>	4	180	28	1	13	3	0	0.149
[(B2246 × B11159) ⊗] ⊗	F3	<i>liCcLLRr</i>	2	150	18	9	52	9	3	0.327
A × [(B2246 × B11159) ⊗]	TC		1	35	0	32	1	0	1	0.935
[(B2246 × B11159) ⊗] ⊗	F3	<i>liCcLlrr</i>	3	161	0	66	13	0	3	0.000
A × [(B2246 × B11159) ⊗]	TC		1	38	27	0	1	1	0	0.394
[(B2246 × B11159) ⊗] ⊗	F3	<i>liCcLlRR</i>	1	33	4	0	52	9	3	0.317
A × [(B2246 × B11159) ⊗]	TC		3	115	62	49	2	1	1	0.457
[(B2246 × B11159) ⊗] ⊗	F3	<i>liCcLlRr</i>	6	359	28	12	208	27	21	0.000
A × [(B2246 × B11159) ⊗]	TC		3	103	13	83	2	1	1	0.000
[(B2246 × B11159) ⊗] ⊗	F3	<i>liCcIlRr</i>	3	125	0	35	52	0	12	0.557
A × [(B2246 × B11159) ⊗]	TC		9	288	0	272	1	0	1	0.796
[(B2246 × B11159) ⊗] ⊗	F3	<i>liCcllrr</i>	9	277	0	72	13	0	3	0.667
A × [(B2246 × B11159) ⊗]	TC		1	33	18	16	2	1	1	0.935
[(B2246 × B11159) ⊗] ⊗	F3	<i>liccLLRr</i>	1	44	0	0	1	0	0	
A × [(B2246 × B11159) ⊗]	TC		1	17	6	5	2	1	1	0.507
[(B2246 × B11159) ⊗] ⊗	F3	<i>liccLlRR</i>	1	51	0	0	1	0	0	
A × [(B2246 × B11159) ⊗]	TC		1	40	21	25	2	1	1	0.673
[(B2246 × B11159) ⊗] ⊗	F3	<i>liccIlRR</i>	1	54	0	0	1	0	0	
A × [(B2246 × B11159) ⊗]	TC		2	58	32	39	2	1	1	0.355
[(B2246 × B11159) ⊗] ⊗	F3	<i>liccIlRr</i>	3	97	1	0	1	0	0	
A × [(B2246 × B11159) ⊗]	TC		4	0	143	129	0	9	7	0.474
[(B2246 × B11159) ⊗] ⊗	F3	<i>iiCcLlRr</i>	4	77	98	88	16	27	21	0.176

^zA, yellow bulb, male-sterile plant with expected genotype *iiCCLLrr* or *iiCCLlrr*.

^yGen., generation; TC, family from testcross of F₂ plants to male-sterile (A) line.

^xNumbers of families (Fam.).

^wObserved (Obs.) and expected (Exp.) ratios of white (W), red (R), and yellow (Y) bulbs. Data were pooled across families after establishing homogeneity of ratios.

^vProbability (*P*) of goodness-of-fit from chi-square analyses.

recessive alleles and B11159 dominant alleles at the *L* and *R* loci.

Amplicons were produced from the promoter regions of *ANS* and *DFR*; however, we never observed any of the polymorphisms reported by Kim et al. (2004a, b, 2005a, b, c, 2006). Amplicons produced from the coding regions of *ANS* and *DFR* were essentially identical to Genbank accessions AY585678 and AY221252 respectively, and SNPs were revealed that segregated in the B2246 × B11159 family. For *ANS*, one T/C SNP was identified at position 358 (Genbank accessions BV722873 and BV722872 for B2246 and B11159 respectively). One C/T SNP in *DFR* segregated at position 257 (Genbank accessions BV722871 and BV722870). Linkage analyses revealed that *ANS* and *DFR* were independently inherited, as expected, because Masuzaki et al. (2006) assigned these genes to chromosomes 4 and 7 respectively of onion. Red bulbs versus yellow bulbs in the B2246 × B11159 family cosegregated with *DFR* and a locus linked at 6.3 cM to *ANS*. Bulb color segregations and SNP genotypes for recombinant families are shown in Table 3. Our results demonstrate that light-red to red bulb color in the B2246 × B11159 family is conditioned by dominant alleles at two loci, consistent with the model proposed by El-Shafie and Davis (1967). One of these loci is identical or tightly linked to *DFR*. The second locus did not cosegregate with *ANS*, but showed linkage to *ANS* at 6.3 cM and was named *L2*.

SEGREGATIONS FOR RED BULBS VERSUS YELLOW BULBS FROM B8667B. Segregations for red bulbs versus yellow bulbs in the B8667 family fit the expected 1:2:1 ratio (13:39:20, $P = 0.394$). Amplicons produced for *ANS* were monomorphic in this family. Amplicons from *DFR* were more than 98% similar to Genbank accession AY221252, and an A/T SNP in *DFR* was identified at position 296 (Genbank accessions BV722868 and BV722869 for red and yellow alleles respectively). Linkage analyses demonstrated that red bulbs versus yellow bulbs cosegregated with SNPs in *DFR*.

SEGREGATIONS FOR RED BULBS VERSUS YELLOW BULBS IN TESTCROSSES OF BYG15-23 × AC43 PROGENIES. We previously

reported that yellow F_2 bulbs from the BYG15-23 × AC43 segregating family occasionally produced red progenies in testcrosses with yellow male-sterile lines (King et al., 1998). An A/T SNP was identified at position 120 of genomic amplicons of *DFR* (Genbank accessions BV722867 and BV722866 and AC43 and BYG15-23 respectively); no SNPs were revealed in *ANS* amplicons. Single nucleotide polymorphisms in *DFR* cosegregated with complementary light-red bulbs in testcrosses of 115 plants from BYG15-23 × AC43. We originally named a locus (*Crb-1* for complementary red bulb-1) for this trait (King et al., 1998); however, this name is not appropriate because a dominant allele at *DFR* conditioned complementary light-red bulbs in this family. Dihydroflavonol 4-reductase mapped to chromosome 7 of onion at 11.1 cM from AOB212 (Martin et al., 2005), in agreement with Masuzaki et al. (2006), who used alien addition lines to assign *DFR* to chromosome 7.

LINKAGE ANALYSES FOR BULB AND SEEDCOAT COLORS IN ONION. We previously described mapping of SSR markers in onion (Martin et al., 2005), of which 22 segregated in the B2246 × B11159 family and nine in the B8667B family. Linkage analyses among the bulb color loci and SSRs revealed that *I* was linked at 21.0 cM to ACM006 (currently unmapped in onion), *B* was assigned to chromosome 1, and *C* to chromosome 6 (Fig. 1). Single nucleotide polymorphisms in *ANS* and *DFR* were not linked to any of the SSRs.

GENETIC MODEL FOR RED BULB COLOR IN ONION. Crosses among populations of yellow bulb onions occasionally produce light-red progenies, for example between North American long-day storage inbreds or short-day Grano types with European Rijnsburger or Brazilian populations (Jones and Peterson, 1952). These light-red hybrids complicate the production of yellow hybrids from crosses among divergent inbred lines. El-Shafie and Davis (1967) proposed that complementary light-red bulbs are conditioned by dominant alleles at the *R* and *L* loci, with deeper red colors occurring when *R* or *L* loci are homozygous dominant. The *L* locus was proposed to be *ANS* (Kim et al., 2005c) and the *R* locus may be *DFR* (Kim et al., 2004b). Hybridizations revealed that *ANS* is single copy, and

Table 3. Parental genotypes and examples of segregations for onion bulb colors and single nucleotide polymorphisms at anthocyanidin synthase (*ANS*) and dihydroflavonol 4-reductase (*DFR*) for F_3 and testcross families from B2246 by B11159.

Family ^z	Gen. ^y	SNP ^x		Genotype ^w	Obs. ^v			Exp. ^v			<i>P</i> value ^u
		<i>ANS</i>	<i>DFR</i>		W	R	Y	W	R	Y	
B11159	P1	T	C	<i>I1ccLLRR</i>	All	0	0	1	0	0	
B2246	P2	C	T	<i>iiCCLlrr</i>	0	0	All	0	0	1	
A × 19100	TC				30	14	24	2	1	1	0.144
19100	F3	C	H	<i>IiCCLlRr</i>	28	7	8	48	9	7	0.221
A × 19124	TC				35	23	26	2	1	1	0.280
19124	F3	C	H	<i>IiCCLlRr</i>	36	8	5	48	9	7	0.897
A × 19136	TC				30	15	14	2	1	1	0.975
19136	F3	C	H	<i>IiCCLlRr</i>	21	9	4	48	9	7	0.103
A × 19142	TC				33	13	17	2	1	1	0.722
19142	F3	C	H	<i>IiCcLlRr</i>	49	4	1	208	27	21	0.153

^zA, yellow bulb, male-sterile plant with expected genotype *iiCCLlrr*.

^y Gen., generation; P, parent; TC, testcross family to male-sterile (A) plant.

^xSNP, single nucleotide polymorphism.

^wGenotype assigned based on observed segregations.

^vObserved (Obs.) and expected (Exp.) ratios of white (W), red (R), and yellow (Y) bulbs.

^u P = probability of goodness-of-fit from chi-square analyses.

Dihydroflavonol 4-reductase (*DFR*) cosegregated with one of the two loci conditioning red bulb color. If anthocyanidin synthase (*ANS*) were the second locus, F_2 plants homozygous for the C allele at *ANS* should not produce red bulbs in the F_3 and testcross families.

Fig. 1. Linkages among simple sequence repeats (ACM) and the *B* and *C* loci in onion. Markers shown in bold text were previously mapped by Martin et al. (2005).

numerous copies of DFR exist in the onion genome (Fig. 2). Our analyses support DFR as the *R* locus of onion; however, duplicated copies of DFR may also control the appearance of red bulbs. In the B2246 × B11159 family, red bulb color did not cosegregate with single-copy ANS, but with a second locus (*L2*) linked at 6.3 cM to ANS on chromosome 4. We propose that yellow bulb onions have been independently selected numerous times from white or red populations and that yellow onions carry independent mutations in structural or regulatory genes controlling the production of anthocyanins. Two of these genes are DFR and ANS, and correspond to the *R* and *L* loci

Fig. 2. (A, B) Autoradiograms from hybridizations of anthocyanidin synthase (A) and dihydroflavonol 4-reductase (B) to gel blots of *EcoRV*-digested DNA from four segregating progenies from the B2246 × B11159 family of onion.

(Kim et al., 2004a, 2005c) respectively. Our results demonstrate that dominant alleles at both DFR and *L2*, an additional locus linked at 6.3 cM to ANS on chromosome 4, also condition red bulb color in onion.

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