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RESEARCH

Mode of inheritance of fruit and stem color of the Italian heirloom 'Rugosa Friulana' Crookneck squash (*Cucurbita pepo*)

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Abstract Crookneck squash, *Cucurbita pepo* subsp. *ovifera*, Crookneck Group, are a familiar produce item in the U.S.A. The Crookneck is an old group of cultivars, almost all of which have yellow young fruits that turn brilliant orange as they age, and dark-green-colored plant stems. 'Rugosa Friulana' (RFR) is an Italian heirloom that is unique among crookneck squash by retaining yellow fruit color throughout development and by having light-colored plant stems. The objective of this investigation was to determine the genetic basis of the persistent yellow fruit color and light stem color of RFR by crossing it with three near-isogenic lines of known fruit- and stem-color genotype. The results indicated that RFR is homozygous for gene *Y*, which confers yellow fruit color, and is unique among edible-fruited subsp. *ovifera* by being homozygous recessive at locus *D*, its *d/d* genotype conferring light-green stem color. The *d/d* genotype was confirmed by diagnostic PCR analysis of the causative *CpAPRR2*-repeated locus. RFR also carries the uncommon top-dominant allele $L\text{-}2^{WS}$, which is partially epistatic to *D* in the stem and epistatic in the fruit and, by inhibiting green and orange coloration, results in the persistence of yellow fruit color through to fruit maturation. RFR segregates for the dominant allele conferring broad fruit stripes, $l\text{-}1^{BSt}$, or for a complementary gene necessary for expression of $l\text{-}1^{BSt}$. Two scenarios are discussed to account for the origin of the recessive *d/d* genotype of RFR. Hopefully, the ancestry of RFR can be determined in the future by whole-genome resequencing and comparative analysis.

Keywords Crop evolution · Crop history · Cucurbitaceae · Fruit-shape diversity · Stem color · Summer squash

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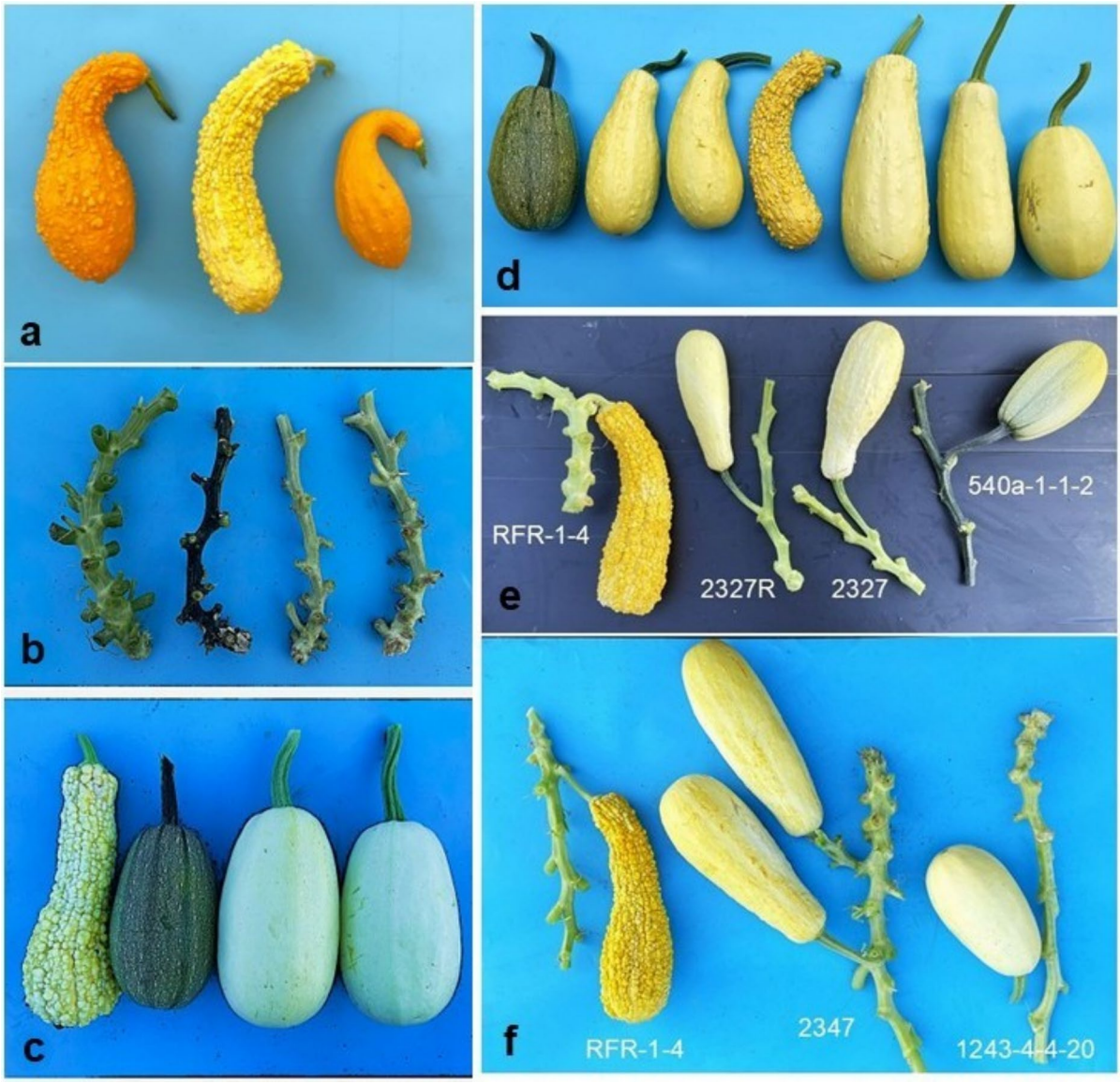
Introduction

Cucurbita pepo L. (Cucurbitaceae) derives much of its economic value from production of its young fruits, known as summer squash. *C. pepo* is native to North America and is generally recognized as containing two cultivated subspecies (Decker 1988). One, *C. pepo* subsp. *pepo*, hails from the southern part of the continent, southern and central Mexico and Guatemala, and was domesticated approximately 10,000 years ago (Whitaker and Cutler 1971;



Teppner 2004; Smith 1997, 2006). The other, *C. pepo* subsp. *ovifera* (L.) D.S. Decker, hails from the central eastern part of the continent, the southern and eastern U.S.A., and was domesticated approximately 5000 years ago (Cowan 1997).

Cultivated *Cucurbita pepo* is extremely variable phenotypically, especially for fruit size, shape, and color (Duchesne 1786). For the species as a whole, the diversity among the edible-fruited cultigens seen at the phenotypic level is probably best understood by focusing on fruit shape (Paris 1986). Accordingly, both of the subspecies contain four fruit-shape groups (Paris 2000a). Subsp. *pepo* contains the Pumpkin, Cocozelle, Vegetable marrow, and Zucchini Groups, but of these only the Pumpkin appears to have existed prior to the year 1492. Subsp. *ovifera* contains the Crookneck, Scallop, Acorn, and Straightneck Groups, and the first three of these, at least, appear to have differentiated long before the arrival of Europeans in North America (Cowan 1997; Paris 2000a).

Crookneck squash have long been a favorite in the United States, especially in the South (White 1856; Rolfs 1916; Bradley and Rhodes 1969; Florida Agricultural Statistics 1992; Schultheis and Walters 1998;

◂**Fig. 1** **a** Fruits, approximately 20 days post anthesis, of three cultivars of Crookneck squash: The yellow fruit of 'Rugosa Friulana' (center), with broad intense yellow stripes alternating with narrow medium yellow stripes with color ratings of 3-4A7 and 3A5, respectively, according to the color guide of Kornerup and Wanscher (1963), and the orange fruits, color 6A7, of 'Early Yellow Crookneck' (left) and 'Yellow Summer Crookneck' (right). Notice the light green peduncle of 'Rugosa Friulana' and the dark green peduncles of 'Early Yellow Crookneck' and 'Yellow Summer Crookneck'. **b** Sections of stems and **c** intermediate-age fruits with peduncles of (left to right) 'Rugosa Friulana' (RFR-1-4) and Accessions 540a-1-1-2, 85a-30-45-17, and 1243-4-4-20. Notice the light stem and peduncle, and medium yellow (non-striped) fruit of 'Rugosa Friulana', the dark stem, peduncle, and dark green fruit of 540a-1-1-2, the light stems, peduncles, and pale green fruits of 85a-30-45-17 and 1243-4-4-20. **d** Intermediate-age fruits (16–20 days post anthesis) of, left-to-right, Accession 540a-1-1-2, F_1 Accession 2327 (540a-1-1-2×RFR-1-4), F_1 Accession 2327R (RFR-1-4×540a-1-1-2), Accession RFR-1-4, F_1 Accession 2331R (RFR-1-4×85a-30-45-17), F_1 Accession 2331 (85a-30-45-17×RFR), Accession 85a-30-45-17. Notice the dark peduncle of 540a-1-1-2 and dark-streaked peduncles of its F_1s and the light peduncles of 85a-30-45-17 and its F_1s, and the light peduncle of RFR-1-4. **e** Stem sections with peduncle and fruit, left to right, of 'Rugosa Friulana' (RFR-1-4), the F_1 Accession 2327R ('Rugosa Friulana'×Accession 540a-1-1-2), the F_1 Accession 2327 (540a-1-1-2×'Rugosa Friulana'), and 540a-1-1-2. Notice the light stem and peduncle of 'Rugosa Friulana', the dark stem and peduncle of 540a-1-1-2, and the dark-streaked light stem and peduncle of the two F_1s, as well as the striping of the fruits of 'Rugosa Friulana', the faint striping of the two F_1s, and the lack of striping in 540a-1-1-2. **f** Intermediate-age fruits with peduncles and stem sections of 'Rugosa Friulana' (RFR-1-4) (left), Accession 1243-4-4-20 (right), and stem section with two fruits and peduncles of their F_1 (Accession 2347, center). Notice the striping of the 'Rugosa Friulana' fruits and the F_1 fruits

Goldman 2004; Loy 2004). The fruits of nearly all cultivars of the Crookneck Group are yellow when young and marketable, 3–5 days post anthesis (dpa). The yellow color is conferred by gene *Y* (Sinnott and Durham 1922), which initiates the change of green to yellow coloration of the fruit on the day of anthesis or shortly thereafter. As they age, yellow crookneck fruits turn a brilliant orange and retain this distinctive color through to fruit maturity. Ordinarily, crookneck squash plants have dark-colored stems and peduncles. The change of fruit color from yellow to orange and the dark stems and peduncles are conferred by the dominant gene *D* (Paris and Nerson 1986), which has been localized to an *APRR2*- (*Arabidopsis Pseudo-Response Regulator 2*-) repeated locus on chromosome 15 of the *Cucurbita pepo* genome. The recessive allele for light coloration, *d*, is reportedly based on a 14 kb deletion within this locus (Zhu et al. 2022). *ARABIDOPSIS PSEUDO RESPONSE REGULATOR2 (APRR2)* genes have been demonstrated to have pivotal roles in regulation of pigment biosynthesis and accumulation in fruit vegetables. Oren et al. (2019) showed that *CmAPRR2* and *ClAPRR2* are causative genes regulating the accumulation of pigments in the fruit rind and flesh of melon and watermelon. Pan et al. (2013) showed that overexpression of an *APRR2* gene in tomato resulted in increased chlorophyll content in immature fruits and a higher level of carotenoids in ripe fruits. Other, more recent studies have also shown the association of *APRR2*-like genes with fruit pigmentation in the Cucurbitaceae (Fan et al. 2025; Liu et al. 2025; Zhai et al. 2025).

Another gene that can affect both fruit and stem color is the multiple-allelic *l-2* (*light fruit coloration-2*) gene, which was recently mapped to a different *APRR2*–repeated locus, on chromosome 5 (Ding et al. 2024; Grebetsadik et al. 2024). The phenotypic expressions of seven alleles have been described for this locus (Paris 2020). Recessive to all others is *l-2*, which confers light fruit color. The most common allele at this locus is *L-2*, which favors color intensification of the fruit surface and interacts with various alleles at locus *l-1* (*light fruit coloration-1*) to confer overall intense (allele *L-1*) color or intensely broad-striped (allele $l\text{-}1^{BSt}$), intensely narrow-striped (allele $l\text{-}1^{St}$), and intensely irregular-striped (allele $l\text{-}1^{iSt}$) fruit coloration, from anthesis through to fruit maturity (Paris 2000b; Paris and Brown 2005). The allele dominant to all others at this locus, designated $L\text{-}2^{WS}$, confers light fruit color, the same as the recessive *l-2* allele. $L\text{-}2^{WS}$ is epistatic to *D* in the fruit and in the stem, conferring light or streaked stem coloration, but may be unstable (Paris et al. 2006, 2013; Paris 2020). Though striping may be absent in young fruits, it can become expressed in intermediate-age fruits of genotypes *l-2/l-2* and $L\text{-}2^{WS}$/— through the complementary interaction of $l\text{-}1^{BSt}$, *l-1St*, or $l\text{-}1^{iSt}$ with the dominant allele, *Pl*, of the *plain light fruit coloration* (*pl*) locus (Paris 1992). A summary description of these five genes affecting fruit and stem color, *D*, *l-1*, *l-2*, *pl*, and *Y*, and their various alleles, is presented in Table S1.

'Rugosa Friulana', also known as 'Bianco Friulano', is an heirloom derived from Italy and a member of the Crookneck Group (Gong et al. 2012).

This cultivar differs from other crooknecks, though, by having fruits that remain yellow through to fruit maturity and by having light-colored stems and peduncles (Fig. 1a). Our objective was to determine the genetic basis of the persistent yellow fruit color and light-green stem of 'Rugosa Friulana'.

Materials and methods

Plant materials used for crossing

Seeds of 'Rugosa Friulana' (RFR) were preserved by the Arche Noah Seed Bank, Schiltern, Austria and kindly provided to us in 2003 by Dr. Tamas Lelley of the University of Natural Resources and Life Sciences, Tulln, Austria. The original seed stock was self-pollinated for two generations, and the inbred used for crossing was designated by us as RFR-1-4.

RFR-1-4 was crossed with three near-isogenic lines of bush growth habit of 'Vegetable Spaghetti' (*Cucurbita pepo* subsp. *pepo*, Vegetable marrow Group) genetic background (Table 1), which had been prepared by backcrossing to 'Vegetable Spaghetti' for six generations followed by selfing and selection for three generations (Paris 2020). All three near-isogenic lines had light green, non-striped young fruits, genotype *l-1/l-1 y/y*. One, of genotype *D/D l-2/l-2*, had dark stems and dark green intermediate-age fruits and was designated 540a-1-1-2. The other two, of genotypes *d/d l-2/l-2* and *D/D L-2^{WS}/ L-2^{WS}* (Paris 2020), had light stems and pale green intermediate-age fruits, the former designated 85a-30-45-17 and the latter 1243-4-4-20. The F_1 plants were self-pollinated or backcrossed to the parents, or testcrossed with a different parent, to obtain F_2, backcross (BC_1), and testcross (TC) progenies.

Field procedures and scoring of phenotypes

Seeds were sown in the field at Newe Ya'ar, northern Israel (32° 43′ 05.4″ N, 35° 10′ 47.7″ E) during April and early May of 2024 and 2025 and the resulting plants were grown using standard cultural practices (Paris et al. 2013). The day of anthesis of one pistillate flower from each parental, filial, backcross, and testcross generation plant was recorded and the fruit that developed from it was scored for color at 3–5 days post anthesis (dpa) at a distance of approximately 50 cm by the first author. The fruit was again scored for color at 16–20 dpa, and stem and peduncle colors were also scored at this juncture. Segregations for fruit and stem colors were subjected to chi-square analysis. Colors of some representative fruits were

Table 1 Accessions used for crossing, their stem and fruit phenotypes and genotypes

Accession (Generation)	Stem	Fruit color		Genotype
		Young, 3-5 dpa	Intermediate, 16-20 dpa	
P_1, RFR-1-4 (S_2)	Light	Medium yellow 2A5**	Striped, intense yellow on medium yellow, or medium yellow 3A7/3A5, 4A7/3A5, 3A5**, ***	To be determined
P_2, 85a-30-45-17 (BC_6F_4) *	Light	Light green 30A3**	Pale green 30A2**	*d/d l-1/l-1 l-2/l-2 Pl/Pl y/y*
P_3, 540a-1-1-2 (BC_6F_4) *	Dark	Light green 29A4**	Dark green 28F6**	*D/D l-1/l-1 l-2/l-2 Pl/Pl y/y*
P_4, 1243-4-4-20 (BC_6F_4) *	Light	Light green 30A3**	Pale green 30A2**	*D/D l-1/l-1 L-2^{WS}/L-2^{WS} Pl/Pl y/y*

RFR-1-4 is a second-generation inbred of 'Rugosa Friulana'

dpa, days post anthesis

*P_2, P_3, and P_4 are sixth-backcross-generation, near-isogenic lines of bush growth habit, derived by using 'Vegetable Spaghetti' as the recurrent parent (Paris 2020)

**Color designations are in accordance with the color guide of Kornerup and Wanscher (1963)

***The fruits of most of the plants were striped, with the color of the stripes closest to 3A7 or 4A7 with the background color being 3A5; some plants had non-striped fruits of color 3A5

quantified according to the color guide of Kornerup and Wanscher (1963), which employs 30 hues, an intensity scale of 2 = palest to 8 = most intense, and darkness (gray contribution) of A = no gray to F = very gray. The hues relevant to the present experiment were 1–4 (yellow, from 1 greenish yellow to 4 orangish yellow), 5–6 (orange), and 28–30 (28 green to 30 yellowish green).

Diagnostic PCR analysis of the *D* locus

To reinforce the interpretation of the results of the genetic segregation at the phenotypic level, the allelic state of the *D* gene was analyzed at the molecular level in eight accessions, the four accessions used for crossing (Table 1) and four others, which acted as genotypic controls. Of the other four, two were the Crookneck squash 'Early Yellow Crookneck' and 'Yellow Summer Crookneck', both of which have dark stems and peduncles (Fig. 1a). For the remaining two, the Crookneck 'Supersett', which has dark stems and peduncles, and the Zucchini 'True French', which has light stems and peduncles, we have high-quality, whole-genome resequencing data.

DNA was extracted from young leaf tissue of all eight accessions, in accordance with the general DNA preparation and procedure described by Fulton et al. (1995). A dominant PCR marker (Fra3) analysis was based on the description of sequence variation at the chromosome 15 *APRR2* locus (Zhu et al. 2022). We used the Fra3 primers proposed by Zhu et al. (2022) (Fra3-forward: GGTCCAAAATAAAATGGCAGTA, Fra3-reverse: AACATCCGAAGTCACTGTGC) for PCR assay. The PCR products were run on 1.2% agarose gel and scored for allelic polymorphism. One µl of the DNA (20 ng) was used for PCR with the 2XPCRBIO HS Taq Mix Red (PCRBIOSYSTEMS), running for 36 cycles, with an annealing temperature of 60 °C.

Results

Young fruit color

The ovaries of all plants were green prior to anthesis. The young fruits of 'Rugosa Friulana', Accession RFR-1-4 (P_1), became completely medium yellow, 1A5-2A5, by 3–5 days post anthesis (dpa) (Fig. S1a). The young, 3–5 dpa fruits of Accession 85a-30-45-17 (P_2), genotype *d/d l-1/l-1 l-2/l-2 y/y*, and Accession 1243-4-4-20 (P_4), genotype *D/D l-1/l-1 L-2^{WS}/L-2^{WS} y/y*, were light green, 30A3. The young, 3–5 dpa fruits of Accession 540a-1-1-2 (P_3), genotype *D/D l-1/l-1 l-2/l-2 y/y*, were also light green but nonetheless slightly more intense green, 29A4, than those of 85a-30-45-17 and 1243-4-4-20.

The young, 3–5 dpa fruits of the F_1 plants resulting from crossing with Accession RFR-1-4 were light green, 30A3 (Fig. S1b). In the F_2 populations, the hue of the young fruits of most of the plants was within the range of yellowish green, hue 30, to greenish yellow, hue 1, according to the color guide. Fruits that were distinctly yellow, hue 2, at 3–5 dpa showed a range in intensity of the color, from medium, as in RFR-1-4, to lighter shades of yellow. However, difficulties were encountered in attempting to classify some 3–5 dpa fruits to hue, as green (29 to 30) or yellow (1 to 2). Specifically, there were fruits that appeared to be transitioning at the peduncle end from green to yellow or that exhibited indefinite striping that was greener or yellower than the rest of the fruit. In such cases, the color was classified to green or yellow pursuant to the majority of the fruit surface. Accordingly, the total F_2 population of the cross between RFR-1-4 and Accession 85a-30-45-17 ($n = 234$) segregated to 196 that were scored as having young green fruits and 38 as having young yellow fruits (Table S2). This result deviated highly significantly from the expected 3:1 single-gene ratio for green to yellow, assuming yellow is recessive in the young fruits (chi-square = 9.578, $P < 0.01$). A similar result was obtained for the total F_2 population derived from crossing RFR-1-4 with Accession 540a-1-1-2 ($n = 253$), which segregated to 211 green and 42 yellow (chi-square = 9.519, $P < 0.01$). However, the F_2 populations adhered to a 13:3 ratio of green to yellow, which assumes that an additional, heretofore unidentified dominant gene is needed, in addition to homozygosity of allele *Y*, for expression of yellow color in the young, 3–5 dpa fruits. The populations of the backcrosses to RFR-1-4, Accession 2346, 2346R ($n = 62$) and Accession 2335 ($n = 111$) did not deviate significantly from the 1:1 single-gene ratio of green to yellow (Table S2). All of the progeny of the backcrosses and testcrosses to Accessions 85a-30-45-17, 540a-1-1-2, and 1243-4-4-20 bore green young fruits.

Stem color

The stems and peduncles of Accession RFR-1-4 were light green (Fig. 1a–f). Those of Accession 85a-30-45-17, genotype *d/d l-1/l-1 l-2/l-2*, were also light green (Fig. 1b–d). The F_1 plants (Accessions 2331 and 2331R, $n=47$) obtained by crossing these two accessions, regardless of the direction of the cross, also had light stems and peduncles. All of the F_2 plants (Accessions 2331-2, 2331-6, 2331Ra-1 and 2331Ra-5, total $n=234$) and the plants resulting from the backcross to RFR-1-4 (Accessions 2346 and 2346R, $n=62$) and to 85a-30-45-17 (Accession 2345, $n=122$) also had light stems and peduncles (Table 2). Evidently, RFR-1-4 and 85a-30-45-17 have in common a gene for light stem and peduncle color.

The stems and peduncles of Accession 540a-1-1-2, genotype *D/D l-1/l-1 l-2/l-2*, were dark green (Fig. 1b–e). All of the F_1 plants obtained by crossing it with Accession RFR-1-4 (Accessions 2327 and 2327R, $n=38$) had stems and peduncles that were light green with dark green streaks, hereafter referred to simply as "streaked". The total population of F_2 plants (Accessions 2327-8 and 2327Ra-7, $n=253$) segregated into three stem phenotypes, dark, streaked, and light, in accordance with a two-gene 3:9:4 ratio of dark: streaked: light (Table 2). Segregation also fit the 3:1 one-gene ratio for dark + streaked: light (chi-square = 0.296, $P=0.58$) and the 3:13 two-gene ratio for dark: streaked + light (chi-square = 0.439, $P=0.50$). The plants of the backcross to RFR-1-4 (Accession 2335, $n=111$) segregated to streaked

Table 2 Segregation for stem color to dark, streaked (light with dark streaks), and light in crossing 'Rugosa Friulana' (RFR) with near-isogenic lines of 'Vegetable Spaghetti' genetic background

Accession/population	Generation	Number of plants				Expected ratio	Chi-square	*P*
		Total	Dark	Streaked	Light			
RFR-1-4	P_1	27	0	0	27	–	–	–
85a-30-45-17	P_2	25	0	0	25	–	–	–
2331	F_1, $P_2 \times P_1$	26	0	0	26	–	–	–
2331R	F_1, $P_1 \times P_2$	21	0	0	21	–	–	–
	F_1, Total	47	0	0	47	–	–	–
2331-2, 2331-6	F_2, $(P_2 \times P_1) \otimes$	117	0	0	117	–	–	–
2331Ra-1, 2331Ra-5	F_2, $(P_1 \times P_2) \otimes$	117	0	0	117	–	–	–
	F_2, Total	234	0	0	234	–	–	–
2346, 2346R	BC_1, $P_1 \times (P_1 \times P_2) + (P_1 \times P_2) \times P_1$	62	0	0	62	–	–	–
2345	BC_1, $P_2 \times (P_2 \times P_1)$	122	0	0	122	–	–	–
540a-1-1-2	P_3	22	22	0	0	–	–	–
2327	F_1, $P_3 \times P_1$	22	0	22	0	–	–	–
2327R	F_1, $P_1 \times P_3$	16	0	16	0	–	–	–
	F_1, Total	38	0	38	0	–	–	–
2327-8	F_2, $(P_3 \times P_1) \otimes$	127	30	63	34	3:9:4	2.764	0.88
2327Ra-7	F_2, $(P_1 \times P_3) \otimes$	126	22	71	33	3:9:4	0.183	0.91
	F_2, Total	253	52	134	67	3:9:4	1.147	0.56
2335	BC_1, $(P_3 \times P_1) \times P_1$	111	0	43	68	1:1	5.631	0.02
2334	BC_1, $P_3 \times (P_3 \times P_1)$	98	50	48	0	1:1	0.041	0.83
2336	TC, $P_2 \times (P_3 \times P_1)$	121	24	31	66	1:1:2	1.810	0.41
1243-4-4-20	P_4	3	0	0	3	–	–	–
2347	F_1, $P_1 \times P_4$	18	0	1	17	–	–	–
2355	TC, $(P_1 \times P_4) \times P_3$	94	0	63	31	1:0	–	–

and light, with no dark-stem plants. The plants of the backcross to 540a-1-1-2 (Accession 2334, $n=98$) segregated to dark and streaked, fitting a 1:1 ratio, with no light-stem plants. These results indicate that RFR-1-4 carries a dominant gene that is partially epistatic to *D* in plant stems.

The stems and peduncles of Accession 1243-4-4-20, genotype *D/D l-1/l-1 L-2^{WS}/L-2^{WS}*, were light green (Fig. 1b,c,f). Those of the F_1 plants obtained by crossing it with Accession RFR-1-4 (Accession 2347, $n=18$) had light green stems and peduncles (Fig. 1f), except for one plant which showed some streaking of the peduncle. When this F_1 was crossed on Accession 540a-1-1-2, genotype *D/D l-1/l-1 l-2/l-2*, none of the progeny (Accession 2355, $n=94$) had dark stems and peduncles, indicating that RFR-1-4 and 1243-4-4-20 have a similar or the same allele, *L-2^{WS}*, that is partially epistatic to *D* in plant stems and peduncles. Of the 94 progeny, 63 had streaked stems and/or peduncles and the remaining 31 had light stems and peduncles (Table 2).

Diagnostic PCR analysis at the *CpAPRR2* locus on chromosome 15

Diagnostic PCR analysis of the four accessions acting as controls confirmed that the three dark-stem, *D/D*, Crookneck accessions, 'Early Yellow Crookneck', 'Supersett', and 'Yellow Summer Crookneck', carry the wild-type marker allele at the *CpAPRR2* locus on chromsome 15 (Table 3; Fig. 2) and the light-stem, *d/d*, Zucchini accession 'True French' carries the deletion at this locus. Among the four parental accessions, the near-isogenic light-stem, *d/d*, Accession 85a-30-45-17 had the deletion at the *CpAPRR2* locus. The near-isogenic dark-stem, *D/D*, Accession 540a-1-1-2 had the wild-type allele at *CpAPRR2*, as expected. And the near-isogenic light-stem *D/D* Accession 1243-4-4-20 had the wild-type allele at the *CpAPRR2* locus on chromosome 15, as its light stem color is conferred by the epistatic *L-2^{WS}* gene. The unique light-stem, *d/d*, Crookneck 'Rugosa Friulana' was confirmed to carry the deletion at the *CpAPRR2* locus (Table 3).

Intermediate-age fruit color

The intermediate-age fruits of Accession RFR-1-4 were medium to intensely yellow, in a range of 3A5 to 4A7. The intermediate-age fruits of Accessions 85a-30-45-17 and 1243-4-4-20 were 30A2, pale yellowish green, and those of Accession 540a-1-1-2 were dark green, 28F6 (Table 1; Fig. 1a, c–f).

The hue of the intermediate-age fruits of filial and backcross populations, for most individual plants, was easier to classify than that of the young fruits, though some doubts were experienced in scoring the pale fruits as yellow (1A2) or green (30A2). The F_1 plants obtained by crossing RFR-1-4 with Accession 85a-30-45-17 (Accessions 2331 and 2331R, $n=47$) and with Accession 540a-1-1-2 (Accessions 2327

Table 3 Accessions subjected to diagnostic PCR analysis, their affiliation to subspecies and Group, stem color, and status at the *CpAPRR2* locus on chromosome 15

Accession	Subspecies, Group	Stem phenotype (and genotype)	*CpAPRR2* status on chromosome 15
Early Yellow Crookneck	*ovifera*, Crookneck	Dark (*D/D*)	Wild-type
Supersett	*ovifera*, Crookneck	Dark (*D/D*)	Wild-type
Yellow Summer Crookneck	*ovifera*, Crookneck	Dark (*D/D*)	Wild-type
True French	*pepo*, Zucchini	Light (*d/d*)	Deletion
P_2, 85a-30-45-17	*pepo*, Vegetable marrow	Light (*d/d*)	Deletion
P_3, 540a-1-1-2	*pepo*, Vegetable marrow	Dark (*D/D*)	Wild-type
P_4, 1243-4-4-20	*pepo*, Vegetable marrow	Light (*D/D*)	Wild-type
P_1, RFR-1-4	*ovifera*, Crookneck	Light (*d/d*)	Deletion

P_2, P_3, and P_4 are sixth-backcross-generation, near-isogenic lines of bush growth habit in the genetic background of 'Vegetable Spaghetti'

RFR-1-4 is a second-generation inbred of 'Rugosa Friulana'

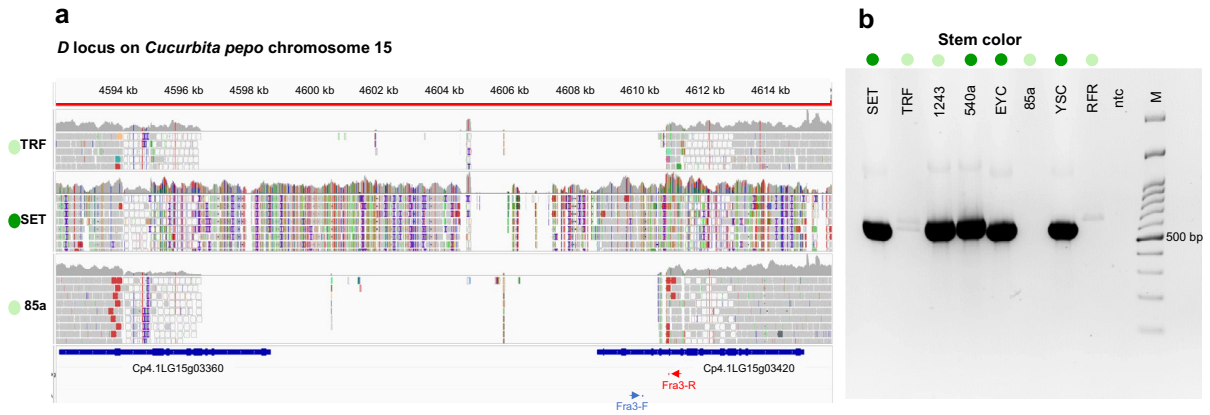


Fig. 2 Genotyping of the *D* locus. **a** Integrative genomics viewer (Robinson et al. 2011) screenshot of sequence comparison for the chromosome 15 *CpAPRR2* locus. Each track represents sequence reads of an accession, TRF = 'True French', SET = 'Supersett', 85a = Accession 85a-30-45-17. Colors are polymorphic sites as compared with the *Cucurbita pepo* reference genome (MU-CU-16 V4.1, Montero-Pau et al. 2018). Dark green and light green ellipses represent the stem color for each particular accession. Gene models are presented as blue horizontal bars below the sequences. Cp4.1LG15g03420 is the *CpAPRR2* gene with a deletion associated with light stem. The positions of the primers for the Fra3 InDel marker are presented. **b** Diagnostic PCR analysis of the *D* InDel marker (Fra3) across 8 representative accessions. Stem color of the tested accessions is indicated by dark green and light green circles above the accession abbreviations. SET = Supersett, TRF = True French, 1243 = 1243-4-4-20, 540a = 540a-1-1-2, EYC = Early Yellow Crookneck, 85a = 85a-30-45-17, YSC = Yellow Summer Crookneck, RFR = Rugosa Friulana, ntc = no template control, M = DNA size marker

and 2327R, $n = 38$), regardless of the direction of the cross, had pale to light yellow fruits, 2A2 to 2A3 (Fig. 1d). The F_2 populations of RFR-1-4 crossed with 85a-30-45-17 (Accessions 2331-2, 2331-6, 2331Ra-1 and 2331Ra-5, total $n = 234$) segregated in accordance with a 3:1 ratio of yellow to green, as expected for a single dominant gene conferring yellow fruit color (Table 4).

A similar result was obtained from crossing RFR-1-4 with 540a-1-1-2 (Accessions 2327-8 and 2327Ra-7, $n = 253$), except that plants having a dark stem, conferred by *D/D l-2/l-2* and *D/d l-2/l-2* genotypes, bore either orange (*Y/—*) or dark green (*y/y*) fruits rather than yellow (*Y/—*) or light green (*y/y*), respectively. Thus, the same gene borne by RFR-1-4 that was dominant and partially epistatic in the stems, $L\text{-}2^{WS}$, was also dominant and epistatic in the fruits. The segregation of combined orange and yellow to dark and light green was in accordance with a 3:1 ratio (Table 4). All 62 plants and all but 1 of the 111 plants of the two backcrosses to RFR-1-4 (Accessions 2346, 2346R, and 2335) bore yellow intermediate-age fruits. The backcross to 85a-30-45-17 (Accession 2345) segregated in accordance to a 1:1 ratio of yellow to green but the backcross to 540a-1-1-2 (Accession 2334) showed a significant deviation, with an excess of orange + yellow as compared with green-fruited progeny. All of the dark-stem plants of this backcross population as well as the testcross population on 85a-30-45-17 (Accession 2336) bore orange or dark green fruits. All of the F_1 plants (Accession 2347) obtained by crossing RFR-1-4 with Accession 1243-4-4-20 bore yellow fruits and the testcross progeny (Accession 2355) of this F_1 on 85a-30-45-17 segregated in accordance to a 1:1 ratio of yellow to green (Table 4).

Fruit striping

The yellow intermediate-age fruits of Accession RFR-1-4 were densely warted over almost the entire fruit surface, thereby partially obscuring a fruit color pattern that would otherwise have been obvious. When examined at the stylar end, where the warting was less dense, the fruits were seen to be boldly striped, with broad intense stripes alternating with narrow medium stripes (Fig. 1a, d–f). These fruits were scored as 3A7/3A5 or 4A7/3A5, meaning broad, intense yellow stripes (3A7 or 4A7) on a narrow-striped, medium yellow (3A5) background. On seven of the 27 observed plants, striping was not discernable (Fig. 1c) and these fruits were scored as 3A5,

Table 4 Segregation for intermediate-age (16–20 days post anthesis) fruit hue in crossing 'Rugosa Friulana' with three near-isogenic lines of 'Vegetable Spaghetti' genetic background

Accession/ population	Generation	Number of plants			Expected ratio	Chi-square	*P*
		Total	Yellow + Orange*	Green			
RFR-1-4	P_1	27	27	0	–	–	–
85a-30-45-17	P_2	25	0	25	–	–	–
2331	F_1, $P_2 \times P_1$	26	26	0	–	–	–
2331R	F_1, $P_1 \times P_2$	21	21	0	–	–	–
	F_1, Total	47	47	0	–	–	–
2331-2,6	F_2, $(P_2 \times P_1) \otimes$	117	93	24	3:1	1.256	0.26
2331Ra-1,5	F_2, $(P_1 \times P_2) \otimes$	117	93	24	3:1	1.256	0.26
	F_2, Total	234	186	48	3:1	2.513	0.11
2346, 2346R	BC_1, $P_1 \times (P_1 \times P_2) + (P_1 \times P_2) \times P_1$	62	62	0	–	–	–
2345	BC_1, $P_2 \times (P_2 \times P_1)$	122	65	57	1:1	0.525	0.46
540a-1-1-2	P_3	22	0	22	–	–	–
2327	F_1, $P_3 \times P_1$	22	22	0	–	–	–
2327R	F_1, $P_1 \times P_3$	16	16	0	–	–	–
	F_1, Total	38	38	0	–	–	–
2327-8	F_2, $(P_3 \times P_1) \otimes$	127	96	31	3:1	0.024	0.89
2327Ra-7	F_2, $(P_1 \times P_3) \otimes$	126	96	30	3:1	0.095	0.75
	F_2, Total	253	192	61	3:1	0.107	0.74
2335	BC_1, $(P_3 \times P_1) \times P_1$	111	110	1	–	–	–
2334	BC_1, $P_3 \times (P_3 \times P_1)$	98	59	39	1:1	4.082	0.04
2336	TC, $P_2 \times (P_3 \times P_1)$	121	61	60	1:1	0.008	0.93
1243-4-4-20	P_4	3	0	3	–	–	–
2347	F_1, $P_1 \times P_4$	18	18	0	–	–	–
2355	TC, $(P_1 \times P_4) \times P_3$	94	42	52	1:1	1.064	0.30

*Orange fruits were borne by dark-stem plants of genotype *D/— l-2/l-2 Y/—*; such plants were obtained in the F_2 progenies (Accessions 2327-8 and 2327Ra-7), the BC_1 progeny (Accession 2334) and the TC (testcross) progeny (Accession 2336); all F_1 plants (Accessions 2327 and 2327R) had yellow fruits, resulting from genotype *D/d* $L\text{-}2^{WS}$*/l-2 Y/y*

medium yellow (Table S3). The fruits of the same age of Accessions 85a-30-45-17 and 1243-4-4-20 were 30A2, pale yellowish green, and those of Accession 540a-1-1-2 were 28F6, dark green, respectively, and none were striped (Fig. 1c–f). Apparently, RFR-1-4 was either segregating for the allele for broad-striped fruits, $l\text{-}1^{BSt}$, or for a complementary gene, such as *Pl*, that was necessary for striping to be expressed. The warting of the fruits of the F_1 plants derived by crossing Accession RFR-1-4 with the near-isogenic accessions was much less dense than in RFR-1-4 itself, and did not obscure striping (Fig. 1d–f). The intermediate-age fruits of all plants of the F_1 populations, 2327, 2331, and 2347, derived by crossing RFR-1-4 with the three near-isogenic lines, exhibited striped fruits (Table S3). However, as compared with RFR-1-4, the striping was less bold or even faint. The fruits of the F_1s derived by crossing RFR-1-4 with 85a-30-45-17 and 540a-1-1-2, 2331 and 2327, were faintly striped, light yellow (2A3) on a pale yellow (2A2) background (Fig. 1d,e). Those of the F_1 derived by crossing RFR-1-4 with 1243-4-4-20 were more obviously striped, medium yellow (2A5) on a pale yellow (2A2) background (Fig. 1f).

In the backcross populations to RFR-1-4 (Accessions 2346 + 2346R and Accession 2335), heterozygosity of inbred RFR-1-4 for $l\text{-}1^{BSt}$ and/or for a gene necessary for its expression could account for the

observed approximately 3:1 segregation of striped to non-striped (Table S3). However, the backcross and testcross populations to the near-isogenic lines (Accessions 2345, 2334, 2336, and 2355), all of which carry allele *Pl* (Table 1), did not conform to the expected 1:1 segregation ratio of striped to non-striped, but instead to a two complementary gene, 1:3 ratio (Table S3).

The F_2 populations derived from crossing RFR-1-4 with the near-isogenic lines would have been expected to have a majority of striped individuals, because RFR-1-4 must have carried allele *Pl* or some other gene necessary for expression of striping. Instead, both F_2 populations had nearly equal numbers of plants bearing striped and non-striped fruits (Table S3). Even if it is hypothesized that three complementary genes are necessary for expression of fruit striping, for an expected ratio of 27 striped to 37 non-striped, the results deviate significantly (Table S3). On some plants, the striping of the fruits was bold but on others only moderately obvious and on yet others the striping was faint and difficult to discern. We are unable to propose a working hypothesis, based on segregation of two or three genes, for the mode of inheritance of striping in the populations derived from crossing with RFR-1-4.

Discussion

'Rugosa Friulana' is an Italian heirloom cultivar of crookneck squash saved from possible oblivion by seed deposition in an Austrian gene bank (Gong et al. 2012). It differs dramatically from all other crookneck squash known to us by having fruits that are persistently yellow from a young age through to fruit maturity and by having light-colored stems and peduncles. We undertook to determine the classical genetic basis for its unique fruit and plant coloration, and observed that four gene loci were mainly accountable.

'Rugosa Friulana' is homozygous for gene *Y*, which confers *Yellow fruit color* (Sinnott and Durham 1922). This is not the gene identified in a recent genomic investigation by Niu et al. (2023), who described a locus causing yellow coloration before anthesis, which is contradictive of gene *Y* and indicative of gene *B* (*Bicolor*) (Shifriss 1961, 1981; Shifriss and Paris 1981). In *Y/Y* homozygotes, the young fruits change from green to yellow on the day of anthesis to a day or two afterwards. Almost all crookneck cultivars are *Y/Y*. Their fruits are yellow when young and turn orange, 6A7, as they approach intermediate age. Those of 'Rugosa Friulana', though, remain yellow, 3A7-4A7/3A5 if striped (Fig. 1a, d–f) or 3A5 if not striped (Fig. 1c). The fruits of *Y/y* heterozygotes are green when young and turn orange as they approach intermediate age (Shifriss 1949; Nath and Hall 1963; Table 4). Although the F_2 data support the need for an additional, unnamed dominant gene for *Y* to be expressed in young fruits (Table S2), there was no possibility of confirming this result in the backcrosses. Possibly, this result may have been reflective of an ephemeral, transitional period during which the young fruits were actively turning from green to yellow. A result more in accordance with the 3:1 expected ratio might have been obtained had the fruits been scored merely two or three days later, at 6 to 8 dpa.

'Rugosa Friulana' is homozygous recessive, *d/d*, for the *Dark stem* gene (Paris and Nerson 1986) (Table 2). All of the other crookneck cultivars that we are familiar with have dark stems and peduncles, and thus are presumably homozygous dominant *D/D*. This was confirmed molecularly by observing that the deletion in the *CpAPRR2* locus on chromosome 15 that results in light stems (Zhu et al. 2022) occurred in 'Rugosa Friulana' as well as in the light-stem, *d/d* 'True French' and Accession 85a-30-45-17, whilst the dark-stem, *D/D* crooknecks 'Early Yellow Crookneck', 'Yellow Summer Crookneck', and 'Supersett', and the *D/D* Accessions 540a-1-1-2 and 1243-4-4-20, did not have this deletion (Fig. 2; Table 3).

'Rugosa Friulana' is also homozygous for the dominant $L\text{-}2^{WS}$ allele, which is epistatic to gene *D* in the fruit and partially epistatic in the stem and peduncle (Paris 2020). This allele has been identified as a large fragment deletion of another *CpAPPR2* gene that is situated on Chromosome 5 (Ding et al. 2024; Grebetsadik et al. 2024). The *l-2* gene is complex, and we did not find the marker described by Ding et al. (2024) to be highly predictive across a set of unrelated accessions. Nonetheless, when the F_1 (Accession 2347) obtained from crossing RFR-1-4 with Accession 1243-4-4-20 ($D/D\ L\text{-}2^{WS}/L\text{-}2^{WS}$) was testcrossed for allelism on the *D/D l-2/l-2* Accession 540a-1-1-2, all of the testcross progeny, Accession 2355, had streaked or light-colored stems and pale-colored fruits (Table 2; Fig. 1f), indicating that 'Rugosa

Friulana' carries the same or a similar mutant allele as the one carried by 1243-4-4-20, *L-2*^{WS} (Paris 2020). All plants of 2355, though, would have been expected to have streaked stems, as all would have had genotype *D/D* or *D/d*. Strangely, approximately one-third showed no streaking. Possibly, the allele at the *l-2* locus carried by RFR-1-4 is different from that carried by 1243-4-4-20, the latter having a greater epistatic effect in the stem, to the point of resulting in entirely light, non-streaked stems. However, if epistasis was the only underlying cause of this result, the segregation in the Accession 2355 population would have been 1:1 streaked to light. The segregation was, in fact, 63 streaked: 31 light (non-streaked) (Table 2), deviating highly significantly from the expected 1:1 ratio (chi-square = 10.894, $P < 0.01$). On the other hand, we also observed that the degree of streaking differed among individual plants of *D/—* *L-2*^{WS}*/—* genotype, not only in 2355 but also among plants of the F_1 and BC_1 generations obtained from crossing RFR-1-4 with 540a-1-1-2 (Fig. 1e). Possibly, variability in the expression of streaking might even have extended to the point of incomplete penetrance.

'Rugosa Friulana' carries allele *l-1*^{BSt}, which confers broad, contiguous striping on the fruit surface (Paris 2000b). Not all plants of 'Rugosa Friulana' bore striped fruits (Fig. 1c), suggesting that it was segregating for *l-1*^{BSt} or for another gene necessary for expression of *l-1*^{BSt}. Striping was not expressed in the young fruits (Fig. S1a,b) but became apparent as the fruits approached intermediate age (Fig. 1a, d–f). In the progenies of the crosses between RFR-1-4 and the near-isogenic lines, at least three other genes appear to have affected the expression of fruit striping, one of which, *Pl* (Paris 1992), is carried by the near-isogenic lines (Tables 1; S1; S3).

Even though the warting of the fruits of 'Rugosa Friulana' is denser than nearly all other crookneck cultivars which we have observed (Fig. 1a), we found that its wartiness is nonetheless conferred by a single incompletely dominant gene (data not presented), as in other cultivars (Sinnott and Durham 1922). The wartiness, though, was decidedly less dense on fruits of the heterozygous progeny (Fig. 1d–f).

Using analysis of data for simple-sequence-repeat loci, 'Rugosa Friulana' has been seen as firmly rooted in the Crookneck Group (Gong et al. 2012). Also, it is similar to the other extant crooknecks by being *Y/Y*. Among the crooknecks, though, the genotype of 'Rugosa Friulana' is unique, differing from all others in three of the most important gene loci affecting fruit color, *D*, *l-1*, and *l-2*. Combined, the data suggest that the fruit-color genotype of 'Rugosa Friulana' is *d/d* *l-1*^{BSt}*/—* *L-2*^{WS}*/L-2*^{WS} *Y/Y* (Tables 2; 4; S2; S3).

Besides the crookneck cultivars, a few acorn and scallop cultivars have yellow young fruits, genotype *Y/Y* (Shifriss 1949; Nath and Hall 1963). Many acorns and scallops also have broad, contiguous striping, genotype *l-1*^{BSt}*/l-1*^{BSt} (Nath and Hall 1963). Striping is not present in any other extant crookneck known to us besides 'Rugosa Friulana', but a green-colored striped crookneck was listed in the late nineteenth century (Goff 1888; Craig 1896).

All heretofore observed edible-fruited cultivars of *Cucurbita pepo* subsp. *ovifera* have dark stems, except for a few cultivars of the Scallop Group. These scallop cultivars have light or streaked stems because they carry allele *L-2*^{WS}, which is epistatic to *D* in both the fruit and the stems (Paris et al. 2013; Paris 2020). Allele *L-2*^{WS} inhibits accumulation of green and orange coloration, but not yellow coloration, of the fruit surface (Paris 1995). Some of these scallops also have ivory white fruit color because, in addition to the suppression of green and orange color caused by allele *L-2*^{WS}, they also carry the dominant *Wf* (*White flesh*) allele (Sinnott and Durham 1922), which suppresses yellow fruit color (Paris 1995). White fruits result from the suppression of all three colors, green, orange, and yellow. Like other Crookneck, Acorn, and many of the other Scallop cultivars, though, 'Rugosa Friulana' has genotype *wf/wf*, which allows yellow coloration to accumulate. As allele *L-2*^{WS} suppresses green and orange, but not yellow, the fruits of 'Rugosa Friulana' can develop an intense yellow coloration without turning orange (Fig. 1a, d–f). This differential color effect favoring yellow coloration and blocking green and orange coloration by the top dominant *L-2* alleles, *L-2*^{WS} and *L-2*^{W}, was applied recently to breed new yellow-fruited ornamental pumpkins, *C. pepo* subsp. *pepo*, Pumpkin Group, such as 'Mellow Yellow' (Gula 2019).

The Crookneck, Scallop, and Acorn Groups are derived from the same geographical region, the central and southern United States (Decker 1988; Cowan 1997), and are thought to be three long-established Groups of subsp. *ovifera* (Gong et al. 2012). The crooknecks are characterized by being heavily warted (Fig. 1a) and such warted squash were grown by

Native Americans 1500 years ago (Cowan 1997). European botanists of the Renaissance were apt to depict realistically new and unusual plants and, although scallops and acorns were repeatedly illustrated in late 16th-century botanical tomes, the crooknecks are absent except for one possible composite image that has been found from that time, but has not been identified with absolute certainty (Paris 2000a). The first item described by Bauhin (1651) under the heading *Cucurbita aspero folio flore luteo semiarcus in morem intorta*, reported as seen by him in 1607, might have been a crookneck squash. Duchesne's sketch no. 83*, drawn ca. 1770, is an early depiction of a crookneck squash (Paris 2007). In the U.S.A., a letter dated 1807 mentioned a cultivar of "long crooked & warted Squash" that had been cultivated in New Jersey "for near a century" (Paris 2000a). Soon after, the Italian horticultural scientist Filippo Re (1811) mentioned a *Zucca a Nodi*, a noduled squash, which he stated to be an early variety from Tuscany (Lust and Paris 2016). Pichat (1866) listed a yellow *Zucca Rognosa Lunga*, *gialla e talora verdastra*, a yellow sometimes greenish, rough, long squash, explaining the name was due to its having protuberances. As for 'Rugosa Friulana', *Rugosa* indicates 'rugged' or 'warted' and *Friulana* suggests that it was grown and/or marketed in Friuli, northeastern Italy.

There are at least two possible scenarios for the origin of this most unusual heirloom cultivar with particular regard to its light stem, *d/d* genotype (Table 2; Fig. 2). The simplest of these would be mutation to *d* in a crookneck cultivar. However, 'Rugosa Friulana' is also phenotypically and genotypically unique among cultivars of the Crookneck Group, and summer squash cultivars of subsp. *ovifera* in general, by its single-flowering trait, which is conferred by the dominant *Mf* allele of the *multiple-flowering* gene, *mf* (Paris and Gur 2022). A detailed sequence-based analysis of 'Rugosa Friulana' with > 40 diverse accessions of *Cucurbita pepo* confirmed that this cultivar has the *Mf/Mf* (single-flowering) genotype whilst the other crooknecks, including 'Early Yellow Crookneck' and 'Yellow Summer Crookneck', have the sequence variant corresponding with the *mf/mf* (multiple-flowering) genotype (Tzuri et al. (2026). The multiple-flowering trait is carried by all other subsp. *ovifera* summer squash but was not present in any subsp. *pepo* until its recent introgression from subsp. *ovifera* (Paris and Hanan 2010; Paris et al. 2012; Tzuri et al. 2026). Another possible scenario would be origin via contamination of a crookneck cultivar by some other, *d/d Mf/Mf C. pepo*. For such a case, a prime suspect would be a squash of the Cocozelle Group (*C. pepo* subsp. *pepo*) (Paris and Gur 2025), one of which, 'San Pasquale', was mentioned specifically by Re (1811). This Group contains many cultivars having light stems, genotype *d/d* and, interestingly, except for the warting, the overall shape profile of 'Rugosa Friulana' is not narrowly necked like other crooknecks (Fig. 1a) but instead resembles that of the cocozelles.

Genomic research of *Cucurbita pepo* has been initiated (Montero-Pau et al. 2017; Xanthopoulou et al. 2019) but generally lags behind that of other important cucurbit species and, although pangenomic research has been conducted for several cucurbit species, it has not yet been reported for *C. pepo* (Shigita et al. 2025). Hopefully, in the not-too-distant future, a thorough resequencing of the *C. pepo* genome will unveil the origin of 'Rugosa Friulana'.

Conclusions

The fruit and plant coloration of 'Rugosa Friulana' is unique among crookneck squash. This cultivar, unlike other crooknecks, has yellow instead of orange mature fruit coloration and light-colored instead of dark-colored plant stems. Like other crooknecks, 'Rugosa Friulana', is homozygous for allele *Y*, which imparts yellow instead of green fruit coloration. The differences in coloration between 'Rugosa Friulana' and other crooknecks are derived from the dominant allele $L\text{-}2^{WS}$, which prevents the conversion of yellow color to orange color over the course of fruit maturation, and the recessive allele *d*, which confers light-colored plant stems. 'Rugosa Friulana' also carries an allele for fruit striping, $l\text{-}1^{BSt}$, which is absent in other crooknecks.

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Data availability The raw data is available from the authors.

Declarations

Conflict of interest The authors declare no competing interests.

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