Genetic Analysis of Quercetin in Onion (*Allium cepa* L.) 'Lady Raider'

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ABSTRACT

Onion bulbs (*Allium cepa* L.) uniform in color, size, shape, and maturity were selected from breeding line 97-50 of the Texas Tech University onion improvement program and were designated breeding line 'Lady Raider'. Bulbs in this line are pearlized, red in color, grano shaped, and are in the short-day onion class, which matures in Lubbock in early summer. In a 1999 grow-out, bulbs of this phenotype were analyzed spectrophotometrically for quercetin content, as measured by total flavonol (TF) content. Quercetin is of interest in the onion industry because of its health-related properties. There had been no previous selection for TF in this line. Values in this parent population were normally distributed and ranged from 79 - 431 mg/kg. Bulbs were grouped by TF concentration into high (>232 mg/kg), medium (203-223 mg/kg), or low (<203 mg/kg) populations and designated P_{OH}, P_{OM}, and P_{OL}, respectively. The populations were caged separately in 2000 and allowed to sib-pollinate, forming three Sib-one (S_1) populations. These populations were designated as Sib-one high (S_{1H}), Sib-one medium (S_{1M}), and Sib-one low (S_{1L}) based upon the parent population from which it was generated. Flavonol concentrations of the P_O populations were compared to those of the S_1's. TF frequencies for all populations were normally distributed and TF ranges differed. TF values of the S_1 populations ranged from 228 - 675 mg/kg. The TF mean of each P_O population was significantly different from that of each S_1 population. Flavonol content in the S_1 generation segregated into classes similar to those of P_O populations, indicating that TF's can be manipulated through selective breeding.

KEY WORDS: flavonols, quercetin, onion, *Allium cepa*, breeding, selection

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Onions are high in phytochemicals, groups of compounds produced by plants that are metabolized but not synthesized by animals (Campbell 1996). Flavonols are a major class of phytochemicals that provide color, texture, and taste (Harborne 1986). The flavonol of interest in this study is quercetin. It is found in many different fruits and vegetables in varied concentrations. Onion (*Allium cepa* L.) ranked highest in quercetin content in a survey of 28 vegetables and 9 fruits (Herrmann 1976, Hertog and Hollman 1996). Quercetin is reported to have protective effects in reducing the risk of cardiovascular disease (Hertog and Hollman 1996, Keli et al. 1996, Knekt et al. 1996), it functions as an anti-cancer agent (Leighton et al. 1992, Knekt et al. 1997) and has promise to be an antioxidant agent (Deschner et al. 1991) because of its antiprostanoid, anti-inflammatory responses and decreased rate of DNA degradation (Formica and Regelson 1995). Quercetin levels tend to be highest in red and yellow onions and lowest in white onions (Patil et al. 1995, Lombard et al. 2002). Amounts of quercetin in onions vary with bulb color, type, and variety (Leighton et al. 1992, Patil and Pike 1995, Lombard 2000).

Regardless of onion bulb pigmentation, quercetin concentration is highest in the outer rings (Patil and Pike 1995). Onion is an intensely selected crop, for which characteristics such as bulb shape, size, color, and single centers are chosen by breeders (Rabinowitch and Brewster 1990). Due to the potential health benefits of quercetin, its importance in onion, and varietal differences, quercetin is a trait of interest in onion breeding programs. There are no reports of breeding for increased levels of quercetin. Here we report our results after one generation of selective breeding for quercetin content in one line of a short-day onion. This information could be useful for breeders of *A. cepa* looking for ways to increase quercetin levels in their crop.

EXPERIMENTAL PROCEDURES

Parental population. In 1997 onion bulbs uniform in color, size, shape, and maturity were selected from breeding line 97-50 of the Texas Tech University (TTU) onion improvement program and formed the breeding line 'Lady Raider'. Bulbs were planted into the field at the TTU Erskine Street Farm Fall 1997 and upon flowering the following spring were mass pollinated in a crossing cage. Seeds were harvested Summer 1998 and January 1999 were sown into flats filled with equal parts greenhouse potting mix (peat:perlite) and sand in the TTU Horticulture Gardens Greenhouse under day temperatures of 25-30°C and night temperatures of 20°C. Flats were moistened with city water until seedlings had two true leaves, whereupon every other watering plants were fertilized with a dilute solution of 20-20-20. Flats were kept moist but not saturated. March 1999 when seedlings were approximately 90 days of age they were transplanted to the TTU Erskine Street Farm. Bulbs were harvested in early summer when 50% of the tops were down. The line was evaluated for phenotype and bulbs with pearlized, rose-red color, grano shape, and in the short-day onion class were selected. Individual bulbs were evaluated for quercetin content following Lombard et al. (2002). Previous studies have shown that 96% of a tissue extract measured spectrophotometrically for TF content (mg/kg) is quercetin (Lombard et al. 2002). We report here levels of quercetin as TFs. Outer, inedible portions from bulbs were removed. Onions were quartered and one quartered section was weighed then frozen in liquid nitrogen. Frozen tissue was ground to a fine powder using a coffee grinder (Braun®, Boston, MA) and quercetin was extracted from 20 g ground onion powder in 80 ml of 80% EtOH by filtering twice through number

8 (Fisher Scientific, Houston, TX) and grade 42 (Whatman, Clifton, NJ) filter paper. Filtrate was collected into 2 ml Eppendorf tubes and stored at -20°C until analysis. Ethanolic extracts were thawed, vortexed, and diluted 10:1 with 80% EtOH to a total of 5 ml. Absorbance (AU) readings were made in duplicate at 362 nm using a Spectronic Genesys 5 (Waltham, MA) spectrophotometer and recorded as mg/kg total flavonols (TF). These onions formed the initial population (P_o). Individuals from the P_o were grouped into one of three categories based upon the TFs observed: high (> 230 mg/kg), medium (208 – 230 mg/kg), or low (< 208 mg/kg).

Selected populations. Bulbs from each sub-population were kept in their respective groups and planted back to the field fall 1999. Upon flowering, bulbs from each population were mass pollinated separately in crossing cages, creating seed for three sibbed (sister or sibling) (S_1) populations. Summer 2000 seed from the sibbed onions were harvested separately and, based on their parentage, were designated as sib population S_{1H} (high), S_{1M} (medium), or S_{1L} (low). This seed was sown in the TTU Horticultural Greenhouse January 2001 and transplanted to the TTU Erskine Farm (Lubbock, TX) spring 2001 as above. Sib-one bulbs were harvested in early summer, selected for phenotype and analyzed as were the previous populations following Lombard et al. (2002) with the following adjustment: rather than quartering the onions, three core samples approximately 4 mm x 6 mm each, were taken from equal distant locations on the perimeter of the onion. Coring minimizes the rotting that follows larger, quartered sampling. Smaller samples still provided adequate tissue for accurate spectrophotometric readings, while increasing the likelihood of more bulbs surviving replanting in the field for vernalization and larger populations the following spring. Means within and between parent and sib-one populations were analyzed using a pooled t-test and corrected pooled t-value.

RESULTS AND DISCUSSION

Values of TFs in the three P_o ranged from 79 - 431 mg/kg and when plotted, followed a normal distribution (not shown). TF values of the S_1 populations ranged from 228 - 675 mg/kg (Table 1).

Table 1. Total flavonols of parental (P_0) and 1st sibbed (S_1) generation progeny selections of 'Lady Raider' onion.

Population	Sample Size	Total Flavonols (mg/kg)		
Parental		Range	Mean	Std. Error
P_{OL}	22	79 – 195	142	6.81
P_{OM}	6	203 - 223	219	3.57
P_{OH}	11	232 - 431	302	17.63
Sib-one				
S_{1L}	92	228 - 445	287	6.02
S_{1M}	30	228 - 565	392	15.47
S_{1H}	43	272 - 675	456	15.44

When TFs of the S_1 populations were plotted against their P_o parental population, the values for TFs had shifted but were clearly distinguished in the three populations. Some of the shift in TF values from the parental to sibbed progeny may be attributed to genetic variation, but the shift is most likely due to the tissue from which the extract came. Tissue analyzed from the P_o populations was taken from a quartered sample extending into the innermost rings of the bulb, including the inner rings, while tissue from the S_1 progeny came from the outermost scales only (after removal of outer dry skin), hence the tissue sampled likely had relatively higher quercetin levels. Quercetin has been reported to be in highest concentration in the outer rings (Patil et al. 1995). However, all individuals from S_1 populations were treated alike and segregated into three distinct groups as in the parental populations. From the P_0 population with the lowest TFs (P_{1L}) was generated the lowest S_1 population (S_{1L}), likewise from the medium P_0 population (P_{0M}) was generated the medium levels of the S_1 population (S_{1M}), and from the P_0 population with the highest levels of the (P_{0H}) was generated the highest levels of the S_1 populations (S_{1H}). TF values observed in the sib-one population, 228 mg/kg to 675 mg/kg, exceeded those of the parent population (range 79 mg/kg to 431 mg/kg). T-tests revealed means of sibbed populations were significantly different from each other and from the parent population (Table 2).

Table 2. Calculated t- values for means of total flavonols within and between parent and sib-one populations of 'Lady Raider' onion * values significantly different (P<0.05).

	P_{OL}	P_{OM}	P_{OH}	S_{1L}	S_{1M}	S_{1H}
P_{OL}		4.66*	10.19*	47.55*		
P_{OM}	4.66*		2.77*		4.05*	
P_{OH}	10.19*	2.77*				4.82*
S_{1L}	47.55*				7.69*	12.35*
S_{1M}		4.05*		7.69*		2.84*
S_{1H}			4.82*	12.35*	2.84*	

Flavonol concentration means for the sib-one populations mirror those of the parent populations. S_{1H} has the highest TF flavonol mean (456 mg/kg), S_{1M} mid-range (329 mg/kg), and S_{1L} the lowest (286 mg/kg).

Onions are an out-crossing species and heterozygous at many loci. Varietal differences (Leighton et al. 1992) and high coefficients of variation in quercetin levels between onion varieties (Lombard 2000) have been reported. Spectrophotometrically obtained total flavonol (TF) data from parent and sib-one populations of 'Lady Raider' reveal continuous, quantitative data and from these data we suggest that TFs are governed by more than one gene. As such, quercetin in onion is likely highly heritable and selection for TFs for increased levels can be achieved.

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