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Phenotypic Variation in Free Folic Acid Content among F_1 Hybrids and Open-pollinated Cultivars of Red Beet

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Abstract. **The importance of folic acid in the human diet has been recognized in recent years by major increases in government recommended allowances. Red beet (*Beta vulgaris* L.) is an important vegetable source of folic acid, however little is known about the extent of variation for native folic acid content in red beet germplasm. A total of 18 red beet entries, including 11 hybrids (F_1) and seven open-pollinated cultivars (OP), were evaluated for free folic acid content (FFAC) in replicated field experiments during 1993 and 1994. Significant differences among entries were detected in all studies. FFAC ranged from 3.3 to 15.2 $\mu g \cdot g^{-1}$ on a dry mass basis. A significant entry × year interaction was detected. Changes in rank of entries between years were minimal among F_1 hybrids, while the changes in rank among OP cultivars were large. These data demonstrate significant variability among cultivated red beet germplasm sources for FFAC. Entries with high FFAC may be useful for increasing levels of this vitamin in red beet.**

Folic acid, also known as pteroylmonoglutamic acid or folate, is a B vitamin (Ockerman, 1991). It plays many important roles in human health, including prevention of neural tube defects, anemia, heart attack, and certain cancers (Czeizel and Dudas, 1992; Mason 1994; McGanity, 1990; Schorah and Smithells, 1991; Sharman, 1982; Werler et al. 1993). Government mandated folic acid supplementation of foods such as flour will begin in the United States in 1998.

Lactobacillius casei is commonly used as a test organism for evaluating folic acid content in foods (Baker et al., 1959). This bioassay measures bacterial density after incubation in a defined medium. The folate available to *L. casei* without pre-incubation with conjugase is usually referred to as free folic acid content (FFAC), which is the only active form that the human body will directly absorb from the diet. Total folic acid consists of FFAC plus those polyglutamates available to *L. casei* after incubation with conjugase (Hoppner et al., 1972).

Most dark-green leafy vegetables are good sources of folic acid, however some species have a higher folic acid content than others. Hurdle et al. (1968) detected a 10-fold difference in folic acid content among four vegetable crops, in which raw cabbage exhibited the highest level (2.40 $\mu g \cdot g^{-1}$, dry mass basis) and raw potato exhibited the lowest (0.29 $\mu g \cdot g^{-1}$, dry mass basis). Leichter et al. (1978) reported FFAC varied from 47.1 $\mu g \cdot 100\ g^{-1}$ (fresh mass basis) in raw cauliflower to 161.5 $\mu g \cdot 100\ g^{-1}$ (fresh mass basis) in raw asparagus in a comparison of six vegetable crops. In a later study, these workers reported FFAC variation ranged from 39.0 $\mu g \cdot 100\ g^{-1}$ (fresh mass basis) in cabbage to 162.3 $\mu g \cdot 100\ g^{-1}$ (fresh mass basis) in spinach among seven vegetable crops (Leichter et al., 1979). Red beet, compared with other vegetables, such as carrot, green bean, cauliflower, asparagus, cucumber, eggplant, pepper, pea, squash, and sweet potato, has a comparatively high free and total folic acid content in raw or cooked form (Goddard and Matthews, 1979; Hoppner et al., 1972; Lakshmiah and Ramasastri, 1969; Perloff and Butrum, 1977). Hoppner and his colleagues (1972) observed variation from 32.4 to 88.7 $\mu g \cdot 100\ g^{-1}$ (fresh mass basis) in FFAC and from 52 to 118 $\mu g \cdot 100\ g^{-1}$ (fresh mass basis) in total folic acid content among four red beet samples. Wang and Goldman (1996) found large phenotypic variation for FFAC among red beet PI accessions (4.4-fold difference between highest and lowest FFAC accession) and inbreds (5.6-fold difference between highest and lowest FFAC inbreds). Despite documentation of FFAC variability among and within vegetable species, little work has been conducted to quantify variation among red beet cultivars for the purpose of characterizing germplasm. Our goal was to evaluate the variability of FFAC among red beet F_1 hybrids and open-pollinated (OP) cultivars.

Materials and Methods

Eleven F_1 hybrids and seven OP cultivars (hereafter designated "entries") were planted in three replications of a randomized

Table 1. List of 18 red beet entries, their type, and origin.

Entry	Type	Origin
Avenger	F_1[z]	Harris Moran
Big Red	F_1	Chriseed
Cx0113	F_1	Chriseed
Cx0115	F_1	Chriseed
CxA9026	F_1	Chriseed
CxA9027	F_1	Chriseed
Red Ace	F_1	Chriseed
Warrior	F_1	Harris Moran
Rosette	F_1	Asgrow
Nun8525	F_1	Nunhems
Pace Maker III	F_1	Chriseed
Pronto	OP[y]	Bejo Zaden
Wonder	OP	Asgrow
RedPak	OP	Asgrow
Detroit Dark Red	OP	Asgrow
Ruby Queen	OP	Asgrow
Cylindra	OP	Chriseed
Cynor	OP	Chriseed

[z]F_1 = F_1 hybrid.
[y]OP = open pollinated.

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complete-block design in 2 years. Entries were chosen from different sources (Table 1) and were sown in single-row plots 4 m long at the Arlington Experimental Station, Arlington, Wis., in 1993. In 1994, the same entries were planted in single-row plots 4 m long at the Walnut Street Garden, Madison, Wis. At harvest, five individual roots of each entry were randomly selected from each block for FFAC determination in each year.

FFAC was determined by the microbiological method described by Baker et al. (1959), Baker and Frank (1967), and Hoppner et al. (1971) with modifications.

Stock culture of test organism. The test organism used in all experiments was *L. casei* subspecies rhamnosis (ATCC 7469) from the American Type Culture Collection Laboratory, Parkville, Md. Freeze-dried cultures of *L. casei* ATCC 7469 were revived in Bacto-SRM broth tubes and incubated at 37 °C for 20 to 24 h. Cultures were centrifuged under aseptic conditions, and the supernatant was aliquoted into 1 milk : 1 Bacto-casei broth mixture and stored at –80 °C as stock culture.

Preparing inoculum and assay media and folic acid standard working solution. Preparation of inoculum and assay media and folic acid standard working solution were performed according to the standard procedures from Baker et al. (1959 and 1967) and Hoppner et al. (1971 and 1972).

Preparing red beet samples and sample assay set. All sample roots were washed and trimmed by hand. Fifty grams of root tissue (fresh weight) were excised, lyophilized, and ground in a Wiley mill. A 0.2-g powder sample of each entry was dissolved in 10 mL phosphate-ascorbic buffer, autoclaved for 5 min, cooled rapidly, and centrifuged. Ten milliliters suppressant was diluted in 10 mL distilled water, which resulted in the sample solution. The sample assay sets of each entry were composed of three amounts (0.5, 1.0, and 1.5 mL) of sample solution in three assay tubes, respectively. Each assay tube in the set consisted of 2.5 mL assay medium, various amount of sample solution, and correspondent distilled

Table 2. Mean squares (MS) from the analysis of variance for free folic acid content of 18 red beet entries beet in field experiments in 1993 and 1994.

Source	df	MS
Year	1	128.13****
Block/year	4	5.99****
Entry	17	50.45****
OP[z]	6	58.60****
F_1[y]	10	50.60****
Between OP and F_1	1	0.02
Year × entry	17	7.48****
Error	68	0.81
Corrected total	107	

[z]OP = open pollinated.
[y]F_1 = F_1 hybrid.
****Significant at $P \leq 0.0001$.

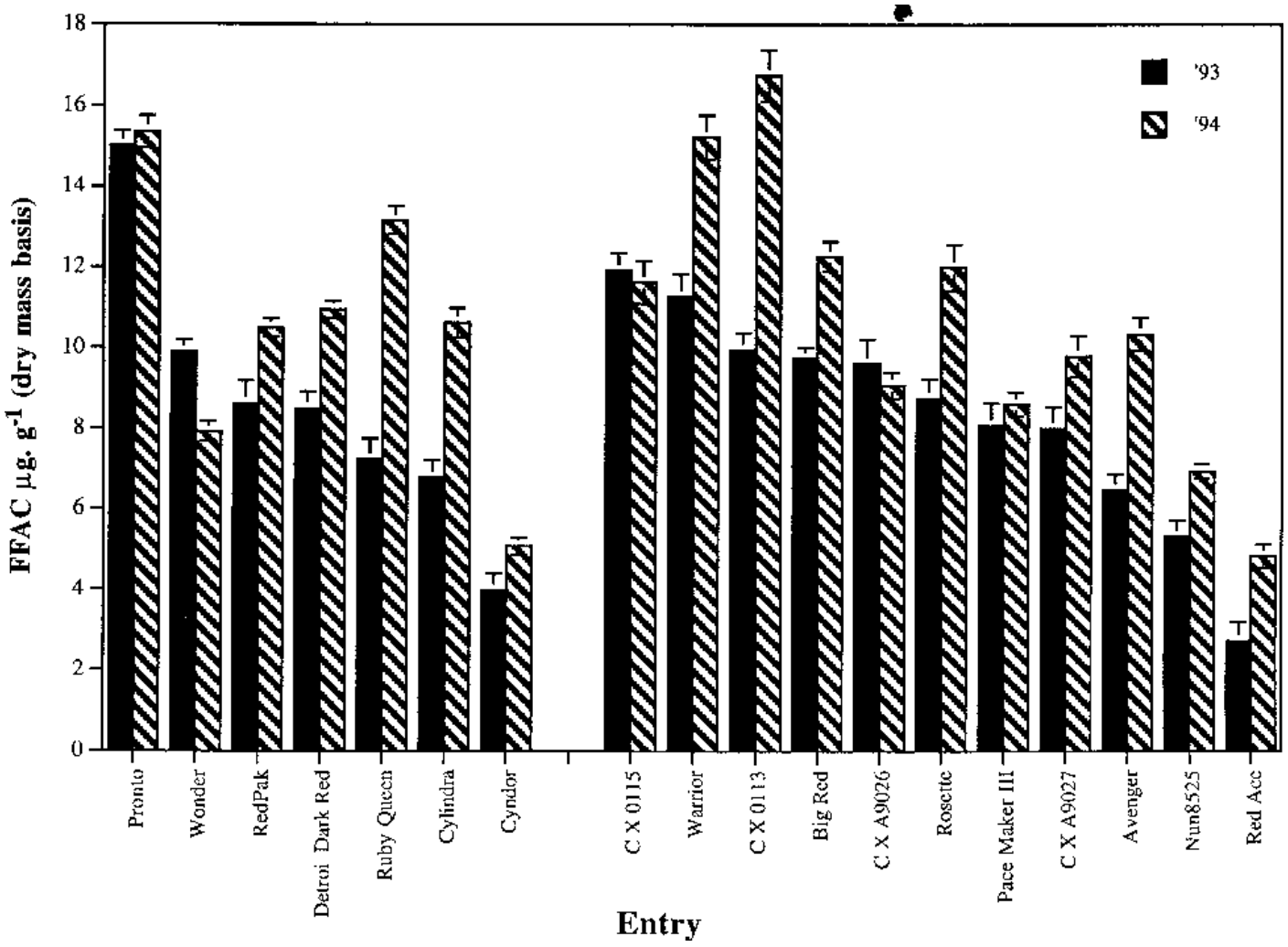

Fig. 1. Free folic acid content (FFAC) of 18 red beet entries (seven open-pollinated and 11 F_1 hybrids) in field experiments in 1993 and 1994 (bars indicate SE).

water to a final volume of 5.0 mL, respectively.

Inoculation and incubation. All assay tubes were covered with plastic caps, autoclaved, and cooled immediately. One drop of standardized (80% transmitance**)** inoculum of *L. casei* was aseptically added to each assay tube, except the blank control, with a sterile syringe using a 22-gauge needle. Tubes were incubated for 20 h in darkness at 37 °C. After the incubation, all assay tubes were boiled for 2 min, shaken vigorously to kill the inoculum strains inside the tube, and cooled for FFAC determination.

FFAC determination. Assay tubes were thoroughly vortexed and suspensions were transferred to optical glassware. The absorbance of each assay tube at 650 nm using a spectrophotometer relative to uninoculated control was recorded. The FFAC of each entry was determined by the regression equation of the standard curve.

Data were analyzed using the generalized linear models procedure of SAS (SAS Institute, Cary, N.C.). Mean comparisons were determined using the least significant difference procedure with $\alpha = 0.05$.

Results and Discussion

The year × entry interaction for FFAC was significant (Table 2). This interaction for F_1 entries was primarily due to changes in magnitude, rather than rank, of the entry means. The interaction for OP entries was, conversely, due to changes in rank (Fig. 1). The OP entries Ruby Queen and Cylindra exhibited greater FFAC in 1994 than in 1993. FFAC in Ruby Queen in 1994 was nearly twice that measured in 1993. These large changes in rank may be due to the inherent heterogeneity in the OP populations. Because of a general lack of genotypic × environment interaction data for productivity traits in red beet in the scientific literature, further investigation will be necessary to determine whether large entry × environment interactions for this trait are common among OP entries.

The mean FFAC of OP and F_1 entries in 1993 [8.43 $\mu g \cdot g^{-1}$ (dry mass basis)] was significantly lower than in 1994 [10.60 $\mu g \cdot g^{-1}$ (dry mass basis)]. This difference in performance between the two years may be due to more favorable growing conditions in 1994. The 1993 growing season was characterized by excessive moisture that resulted in reduced crop growth. Reduced photosynthetic activity would influence plant FFAC due to the association of folic acid synthesis and photosynthesis (Cossins and Shah, 1972).

Highly significant differences among entries were detected, and a large range of FFAC was measured among OP entries and among F_1 entries (Fig. 1). Mean FFAC across all entries ranged from 3.8 to 15.2 $\mu g \cdot g^{-1}$ (dry mass basis). Mean FFAC in OP entries was not significantly different from the mean of F_1 entries; however, the highest FFAC entry was an OP (Pronto) and the lowest was an F_1 (Red Ace). In general, more variation in FFAC was measured among OP entries than F_1 entries. The narrow variation among F_1 entries may be due to 1) fewer F_1 entries evaluated or 2) the narrow genetic base of hybrid red beet compared to OP populations. Most F_1 hybrid red beet cultivars make use of sterile lines developed in or derived from the Univ. of Wisconsin breeding program (W.H. Gabelman, personal communication)

The FFAC of red beet samples analyzed by Hoppner et al. (1972) varied from 32.4 to 88.7 $\mu g \cdot 100\ g^{-1}$ (fresh mass basis). Perloff and Butrum (1977) observed 69 and 38 $\mu g \cdot 100\ g^{-1}$ (fresh mass basis) for FFAC in raw and cooked red beet, respectively. In our investigation, 3.4- and 3.5-fold differences in FFAC were found within OP and F_1 entries, respectively. These results indicate a larger variation than that (2.7-fold) reported by Hoppner et al. (1972), which is primarily due to the increased number of entries and diversity of the entries used in our study. However, the variation is smaller than that among PI accessions and inbreds for FFAC (Wang and Goldman, 1996), which is likely due to a greater level of genetic diversity among PI accessions and inbreds. Our findings indicate that large phenotypic variation for FFAC exists in red beet germplasm, which in turn could be used for further study of the genetic control of this vitamin in red beet.

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