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ANALYSIS, EVALUATION, AND IMPROVEMENT OF FLAVOR TRAITS IN LONG-DAY ONION GERMPLASM

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ANALYSIS, EVALUATION, AND IMPROVEMENT OF FLAVOR TRAITS IN LONG-DAY ONION GERMPLASM

By

Cheng Luo

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ABSTRACT

ANALYSIS, EVALUATION, AND IMPROVEMENT OF FLAVOR TRAITS IN LONG-DAY ONION GERMPLASM

By

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Two important flavor traits, sweetness and pungency were evaluated in long-day, storage onions (*Allium cepa*. L). Sweetness was determined by refractive index for soluble solids (SS) and by HPLC for sugars. Pungency was determined by Schwimmer and Western procedure for pyruvic acid (PA) concentrations in plant tissues. Initial experiments involved screening of 36 onion lines involving 7 inbreds, 6 parental single crosses, 7 MSU experimental hybrids, 9 long-day and 7 short-day cultivars. Significant differences in the percent of SS and sugar, and tissue concentration (μmole per gram fresh tissue weight) ($\mu mol.g^{-1}$) of enzymatically developed PA in the bulbs were detected within the inbred populations and among the hybrids and cultivars. The SS level increased about 2-3% and the PA concentration decreasing about 3-4 $\mu mol.g^{-1}$. in the first two months after storage in response to cold storage. Intraline and interline crosses were made among the plants selected for various SS and PA levels. Selection for maximum change for SS or PA was most effective from high x high or low x low populations. Broad sense heritability and realized

heritability for SS were 84.5 % and 78.6 %, respectively. The levels of heritability for PA were 67.8% and 69.8%. Heritability levels were estimated from the data of the parental, F1, F2, and backcross populations over five years. Frequency distribution for SS and PA, using the data from an experiment of two-way crosses followed by backcrossing, confirmed previous reports of additive dominance of high SS over low SS and low PA over high PA.

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INTRODUCTION

Bulb onions *(Allium cepa* L.) are of major economic and dietary importance in the U.S. and many parts of the world. The U.S. production of bulb onions during the past decade has increased as much as four times of that in the 1970s (Love, 1994). It is predicted that the production and value of the U.S. onions will continue to increase and reach $1 billion by 2000, compared to the $666 million averages for 1991-1993 (Love, 1994). The quality of onions in terms of taste, shape, color, storability, and resistance to pathogen and insect attack has been improved remarkably due to extensive new cultivar development in the past decade (Pike, 1986).

Sweet onions, one of the fresh market onions, always have been in high demand on the market, and the price of sweet onions is generally 25-50% higher than that of storage onions (Smittle, 1988). Most of the production of sweet onions, however, is found in the southern and western regions of the USA, but the poor storability of sweet onions generally restricts them to short-term market sales. They are generally available only during spring and summer. Limited success has been realized with controlled atmosphere (CA) storage to extend

the sweet onion season (Smittle,1988). The major part of the U.S. storable onion production is in the northern and northeastern regions of the USA and these onions are usually available year-round.

In Michigan, onions ranked number one in value for fresh market vegetable production at $16 million on 7,100 acres in 1994 (Fedewa and Pscodna, 1995). This value placed Michigan ninth in the nation for onion production. Most of Michigan's production is storage onions with remarkably pungent flavor. These onions often are referred to as long-day onions. The Michigan onion producers have a long-standing desire to grow their own onions with improved flavor qualities to improve competition with onions produced in other states.

Breeding for onion flavor quality is difficult for several reasons. First, flavor quality is a quantitative trait, and therefore, it cannot be evaluated fully until later generations. Secondly, the onion is a biennial plant which normally requires two years to complete a life cycle, which adds a measure of inefficiency to a breeding program. Thirdly, to fully evaluate flavor quality, several component traits need to be measured, which primarily include sugars and pungency levels. However, the methods available for measuring these flavor components are generally complicated and inefficient for breeding programs. Finally, storability of onions appears to be somehow related to onion flavor. Reduced pungency in onions can lead to a substantial loss of the bulbs after a prolonged period of

storage (Owen, 1950). Lin and coworkers (1995) reported a negative correlation between onion pungency and neck-rot disease index.

A major objective of this study was to develop a system which could be used efficiently in breeding programs for flavor evaluation and improvement of storage onions, and to determine the change and stability of flavor traits, primarily sugar concentrations and pungency in onion parent and progeny populations. Crosses between onions with determined flavor levels were made to study how selection and parent line combination could affect the flavor traits in subsequent generations. Another objective was to determine flavor heritability through genetic analysis based on parent and offspring sugar and pyruvic acid levels. The last objective was to estimate the effects of yearly environment variables on flavor traits in the germplasm studied in this research.

LITERATURE REVIEW

The Onion

Bulb onion is classified taxonomically to the genus of *Allium* under the family of *Alliaceae*. The *Allium* genus contains a large group of more than 600 species (Hanelt, 1986) including many economically important vegetables like garlic, leek, and green bunching onions. *Allium cepa* L. is the botanical name for bulb onion. This group also includes several species with the basic chromosome number x=8, 2n=16, and are mostly diploid (Astly, 1989). The occasional occurrence of individual tetraploid bulbs, however, has been reported (Vosa, 1976).

The bulb onion is one of the oldest cultivated vegetables in the world. Archaeological discoveries date onion culture to at least 2800 B.C. (Vavilov, 1951). Its primary center of origin was central Asia, with secondary centers in the Near East and the Mediterranean region. The onion was one of the first cultivated plants taken to the Americas from Europe. Onion was introdused first to the Americas by Columbus, who took it to the Caribbean. Later, it was imported and established in the northern regions of the U.S. in the early 17th century (McCollum, 1976).

In the U.S. onions generally are classified as 'short-day' or 'long-day' onions in regard to bulbing. Short-day onions are produced in the southern

regions and long-day onions are produced in the northern regions of the U.S. This is an inaccurate classification because all onions are considered to be long-day plants, the various types bulbing differently in response to day length.

The onion is grown widely and is economically important in the U.S. and worldwide. The onion is the third most valuable commercial vegetable in the U.S. ($589 million in 1991), following only tomato and lettuce (USDA, 1991). Production of fresh market onions is concentrated in the southern and western states, whereas storage onions are grown primarily in the northeastern and midwestern regions. Love (1995) predicted that by the year 2000, the U.S. onion industry will produce a $1 billion crop, 50% more than the $666 million average for 1991-93. Globally, the leading producers in 1987 were China (3.6×10^7 MT), India (2.8×10^7 MT) and the USA (2.0×10^7 MT each) (Yamaguchi, 1983).

Breeding and Seed Production

Onion generally is regarded as an outcrossing species, although estimates of the amount of natural outcrossing vary somewhat. Van der Meer and Van Bennekom (1968) reported outcrossing rates in the field in the range of 73 to 100%, whereas under glass the rate varied from 29 to 82%.

As an outcross-pollinating species, onion exhibits severe inbreeding depression upon selfing. Selfing for several generations produces uniform lines, but much vigor is lost in the early generations (Jones and Davis, 1944). Because of the severe inbreeding depression, isogenic lines are uncommon.

Uniform lines for a specific trait can be achieved and vigor maintained by recurrent selection, mass selection, sibbing, or backcross strategies (Barham, 1950; Clark, 1949; Pike, 1986).

Jones and Clark (1943) published a classical paper on the inheritance of male sterility in onion. They demonstrated that male sterility is determined by the interaction of cytoplasmic and nuclear factors. Plants with the sterile 'S' cytoplasm and homozygous for the recessive ms gene were male sterile; plants with any other cytoplasm and gene combination were fertile.

In a typical onion-breeding system, having obtained a number of selected male-sterile and potential pollinator lines, a series of experimental F1 single crosses are made among them and the progenies are evaluated subsequently. The crosses generally are made from flowering plants raised from mother bulbs of the parents, and grown in insect-proof isolation cages. Pike (1986) provided a detailed description of the type of cage and summarized the practical procedures for using it under U.S. conditions.

Flies and bees commonly are used to aid in plant pollination (Van der Meer, 1968). In onion-breeding cages, bees have been found to be better pollinators than flies.

The use of weak inbred parents may result in low seed yield, especially when female plants have small seed heads (Campbell, 1968). Erickson and Gabelman (1958) showed that inbred lines produced on average only about half

of the seed yield of hybrid crosses. A partial solution to this problem has been achieved through development of three-way hybrids, whereby a vigorous male-sterile F1 is used as the female parent with a third inbred as the field pollen parent. This system usually produces significantly higher seed yield than a two-way hybrid. According to Dowker (1989), it is doubtful that if a three-way hybrid has the same potential for heterosis or uniformity as a two-way hybrid.

Onion Flavor Quality

Flavor is one of the most important quality components of onions. People like onions because of their characterisic flavor. Onion flavor generally can be described as 'sweet' or 'bitter', 'mild' or 'hot', and 'mildly pungent' or 'strongly pungent' (Schwimmer and Guadagni, 1962; Pal and Singh, 1988; Randle *et al* .,1993). These terms, however, can be misleading and confusing to the public, and even to the onion bulb producers and breeders. For instance, 'sweet' usually is perceived by people as a sugar-like taste, but a sweet type onion is more likely considered to have a less pungent flavor rather than a sugar-like taste. In fact, in many onion flavor articles, the concern is with pungency rather than sweetness (Randle, 1992; Wall and Corgan, 1992). Many onion researchers have agreed that the characteristic of an onion taste is primarily determined by sulfur compounds and modified by sugars (Plateniu and Knott, 1944; Randle *et al.*, 1993).

Onion Sweetness

The main components of sweetness of onions are the nonstructural carbohydrates including glucose, fructose, and sucrose together with a series of oligosaccharide, the fructans (Suzuki and Cutcliffe, 1989). The nonstructural carbohydrates also account for the major portion of the dry weight. They range from 41% to 88% of the total dry weight in various onion cultivars (Bajai *et al.*, 1980).

Bajai *et al* ., (1980) tested 12 onion cultivars for an assessment of sugar contents. These cultivars included red and white onions that were thought to be different in sugar content. They showed that the reducing sugar content of the 12 cultivars ranged from 12 to 22 g.100 g^{-1} in dry weight, while nonreducing sugars varied between 25 to 62 g. 100 g^{-1}. No differences in reducing sugar were noted between red and white onions.

Starch, which is found commonly in many crops as one of the storable carbohydrate forms, usually is not detectable in onion bulbs. Wilson *et al.*, (1985) reported, however, that foliage of the plants, during growth, may contain starch.

One detectable storage sugar form in onion is a series of fructosyl polymers based on sucrose with varying degrees of polymerization (Bacon, 1957; Darbyshire and Henry, 1979). The fructans can range from 20% to about

50% of the total carbohydrate in the leaf base and later can be hydrolyzed to give an increased fructose concentration in the bulb tissues (Bacon, 1957).

Heat-processed or boiled onions have a characteristically sweet taste. The sweetness is not due to the level of sugars in the onions. Yamanishi and Orioka (1955) found that n-propanethiol increased in concentration during boiling. This compound is 50 to 70 times as sweet as sucrose.

Birth and Dull (1985) studied the composition of the low-dry-matter onion 'Granex' and the high-dry-matter onion 'Creole'. They reported that more than 80% of the dry matter in these cultivars is made up of fiber and sugars. The same information was provided by Darbyshire and Henry (1979) who also found an association of soluble oligosaccharides with dry weight in onion.

Sugar Distribution

Sugar concentration per unit of fresh weight of an onion leaf is lowest at the top and highest in the basal part. Steer and Darbyshire (1979) tested the distribution of sucrose, fructose, glucose and trisaccharides in onion leaf tissue. Their study showed that the basal part was about 6 mg/g higher in sugar content than the top tissues.

Bacon (1957) examined the distribution of the lower molecular weight fructans in onion bulbs and found these compounds to be absent from outer, older leaf bases, and present in increasing amounts from the outer to the inner

leaf bases. Darbyshire and Henry (1978) reported that free fructose concentration was highest in the outer leaf bases and lowest in the innermost. They also observed that fructans and sucrose were high in the inner region, and low in the outer region. Glucose did not vary much across the bulb.

Sugar Determination

1. Soluble solids

Mann and Hoyle (1945) studied the use of a refractometer as a means of determining soluble solids (SS) and dry matter content of onion bulbs. In their results, the refractive index of the onion juice was expressed as a percentage of sucrose concentration. They found the sugar concentration and the percentage dry matter closely correlated ($r = 0.91$). High-dry-matter onions can, therefore, be selected using a refractometer, which is much faster than the oven-drying determination.

2. Individual sugars and total sugar

High pressure liquid chromatography (HPLC) and gas chromatography (GC) can be used for analysis of individual sugar components in plant tissue. Darbyshire and Henry (1978) used HPLC to study the distribution of fructans in onions. They successfully separated fructose, glucose and sucrose from the extraction. A series of fructans with different degrees of polymerization also were separated, but the concentrations were much lower than the other three sugars. Darbyshire and Henry (1979) also later made a comparison among cultivars of low, medium, and high percentage dry matter. The HPLC results showed that

low-dry-matter cultivars contain high levels of glucose, fructose, and sucrose and only trace amount of fructans; whereas, medium-and high-dry-matter cultivars contain lower glucose and fructose concentrations and substantial amounts of fructans.

Genetic Factors Influencing Onion Sugars

Because soluble carbohydrates account for most of the soluble or dissolved solids, most of the published literature related to the genetics of onion sugars deal with soluble solids. An early publication about the heritability of soluble solids in onions was made by Warid in 1952. He used a components of variance method on parents and F2 generations to study the heritability of soluble solids in onions. After crossing onions with high solids with onions with low solids, he concluded that the heritability was 71% from parent-offspring regression. He also estimated that there are 4-10 gene pairs for soluble solids, and that partial dominance of low solids is involved. Later, Owen (1961) examined the segregation of soluble solids genes, and he postulated an additive gene effect in the inheritance of solids.

Many researchers (McCollum, 1968; Kadams and Nwasike, 1986; Lin *et al.*, 1995; and Simon, 1995) reported the heritabilities of solids in several onion populations to be significantly high (60-80%). McCollum (1968) also reported a negative correlation between SS and bulb size.

Simon (1995) investigated the genetics of pungency and SS in long-day onions, using an eight-inbred onion diallel for two years and a four-parent subset of this diallel for one or two more years. He obtained a very high broad sense heritability of 83% for SS. The generation means analysis of four crosses indicated an acceptable fit with a simple additive-dominance model to explain the inheritance of the SS trait.

Environmental and Storage Factors

Randle (1992) reported that sulfur (S) nutrition affected the concentration of total and individual sugars in short-day onion germplasm. On average, the high-S treatment increased sugar content in most of the onions. Bulb dry weight correlated negatively with bulb S concentration. Sucrose and fructose levels changed differently in response to S supply, but no such change was found for glucose.

Steer (1982) examined the effect of day/night temperature on the accumulation of sugars by an onion cultivar. High temperature during both day and night increased dry matter but had no effect on sugars. It was stated that this could be due to the change of water content that simultaneously occurred with the change of temperature. As a result of water changes, dry matter concentration would fluctuate accordingly.

Onion sugar concentrations tend to decrease with prolonged storage (Smittle, 1988). Fractan hydrolysis and fructose increases may occur in onions

after a short period of storage (Darbyshire,1978). However, the ratio of sucrose, glucose and fructose may not be affected by storage methods and duration (Smittle, 1988). Under controlled atmosphere conditions, the decrease of onion sugar concentrations tend to be slower than under cool temperature conditions which commonly are used for onion storage.

Onion Pungency

Lancaster and Boland (1990) summarized that there are over 80 flavor volatile compounds that have been identified from onions. Over 60 of them are S-containing compounds. It is likely that other compounds also are involved, but their detection and contribution has yet to be reported (Schwimmer, 1969).

Onion tissue has no pungent odor until the cells are injured (Lancaster *et al.*, 1989). Onion flavors quickly develop after cell injury. The enzyme alliinase starts hydrolysis of S-alk(en)yl cysteine sulfoxides which are the primary precursors for production of S volatiles associated with flavor and odor. Cultivars that have high levels of the flavor precursors and high alliinase activity have the potential to produce strong pungent flavor upon injuring of the cells. Soil and environmental factors such as soil type, S content, temperature, and irrigation levels also can affect substantially onion flavor development.

1. The precursors

Onion flavor develops from flavor precursor compounds. Four distinct S precursors exist in the genus *Allium* (Lancaster and Boland, 1989). They can

change from nonvolatile into volatile S compounds when the cells are injured, and serve as the base materials that can cause *Allium* plants to have a strong flavor. The four S precursors are:

(1) (+) S-Methyl-L-cysteine sulfoxide

(2) (+) S-Propyl-L cysteine sulfoxide

(3) trans-(+)-S-(L-propenyl)-L-cysteine sulfoxide

(4) S-(2-Propenyl)-L-cystein sulfoxide.

An *Allium* species very often has a flavor distinguishable from other alliums because of the quantitative and qualitative difference in flavor precursor content. Freeman and Whenham (1976 a) measured these precursors in some *Allium* species with gas liquid chromatography. The proportion of S-alk(en)yl radicals (e.g., Methyl, Propyl, and Propenyl) in *Allium* species are different. Onions usually contain S-methyl, S-propyl, and very high S-1-propenyl cystein sulfoxides, but no S-(2-Propenyl) cystein sulfoxide, which is the dominant flavor precursor in garlic.

2. Formation of onion flavor

The enzyme ultimately responsible for the development of onion flavor compounds after cell injury is alliinase, S-alk(en)yl-L-cysteine sulfoxide lyase. Allinase catalyzes the reaction in which the S-alk(en)yl sulfoxide group is eliminated from the substrate during hydrolysis. Two products, the lacrimator

identified as 1-propenyl sulfuric acid and the cysteine, are formed at the end of this reaction:

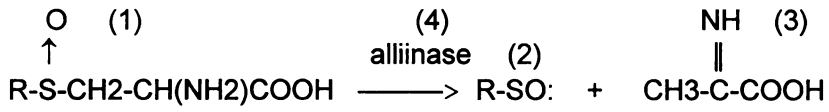

Figure 1. Hydrolysis of flavor precursor in onion (1) S-alk(en)yl cysteine sufoxide; (2) S-alk(en)yl sulfenic acid; (3) cysteine; (4) S-alk(en)yl-L-cysteine sulfoxide lyase.

The two products are very unstable. Sulfenic acids have a half-life of about 90 seconds (Carson *et al.*, 1966), and can undergo nonenzymatic rearrangements to produce a wide range of volatiles that together give the characteristic aroma to an onion. Cysteine can be broken down into ammonia and pyruvic acid in onion homogenates (Lancaster and Boland, 1989). This reaction is described commonly as:

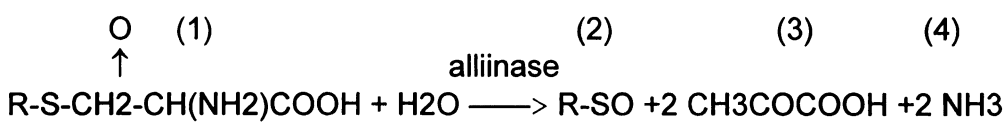

Figure 2. Formation of volatile S compounds in onion after hydrolysis of flavor precursors. (1) S-alk(en)yl cysteine sufoxide; (2) S-alk(en)yl sulfenic acid; (3) pyruvate; (4) ammonia.

When the lacrimator factor, thiopropanal s-oxide, rearranges propanal, carbonyl derivatives of propanal and elemental S are produced. This was proved by Freeman and Whenham (1976 b), who used a synthetic *in vitro* system to demonstrate the importance of thiosufinates as intermediates in the formation of onion volatiles.

Alliinase is found not only in *Allium cepa*, but also in most members of the genus *Allium*. Purification of Allinase has been successful both in garlic and onion by using standard precipitation and chromatographic separation methods (Tobkin and Mazelis, 1979; Lancaster and Boland, 1989).

Besides the sweet and pungent taste, onion may develop a bitter, alkaloid-like taste an hour after homogenization of the tissue. The role of the S-alk(en)yl cysteine sulfoxides and alliinase in the development of this bitterness is not clear, but the development of the bitterness requires an enzyme, since heat-treated or acid-treated onions do not develop bitterness (Schwimmer, 1967). The bitter taste appears at a slower rate than the onion odor and pungency. This could be due to secondary reactions following the initial action of alliinase on S-propenyl cystein sulfoxide (Schwimmer, 1969). The exact compound responsible for the bitterness remains unknown.

Measurement of Pungency

Schwimmer and Guadagni (1962) studied the method of estimating onion flavor by smell. They defined a threshold concentration of onion juice in water

for a smell test. The threshold was the minimum concentration of onion juice diluted with water that could be detected by 70% of the judges. Pungency evaluation by smell alone, however, may not reflect the pungency that would be perceived by taste accurately. Aromatic compounds may be perceived more easily by taste than by smell, because samples are warmed by the mouth, and aromatic constituents become more volatile (Jellinek, 1985).

The measure of pungency by a taste test is considered by some researchers to be very inaccurate, because of the accumulative effect of successive tasting (Platenius and Knott, 1941). Development of chemical methods for quantification and identification of flavor-related substances has made it possible to express the result in numerical values.

Chemical methods of measuring onion flavor

Sampling procedures to estimate flavor potential in onion was studied by Randle (1992). He suggested that a five-bulb sample and four replications were sufficient to detect desired differences for sugars and PA. He also indicated that analysis of S in onions requires a larger sample size and more replications.

The pioneering work of Platenius (1935) on the determination of volatile S compounds involved the application of acid hydrolysis of the onion at high temperatures followed by analysis of S in a distillate.

A thin-layer chromatography method for evaluation and separation of the flavor compounds of onion juice was developed by Luke (1971). This method can measure the relative concentration of other products involved in the action of alliinase on the flavor precursors.

Gas-chromatographic (GC) methods and GC-mass spectrometry have been used successfully in separation and analysis of flavor compounds in onions. Freeman and Mossadeghi (1971) first used GC methods to analyze flavor and odor compounds. They also indicated that this method may alter the amounts of flavor components due to the instability of 1-propenyl compound at the high temperature employed.

By using supercritical carbon dioxide extraction, Sinha *et al.,* (1992) obtained flavor components from onion tissues. GC analysis results showed the presence of 28 S-containing compounds, including diallyl thiosulfinate, propyl methanethiosulfonate, dithin derivatives, diallyl sulfide, diallyl trisulfide, and six other compounds.

Bennet (1945) first qualitatively detected the presence of relatively large amounts of PA in onions. Morgan (1946) proved its presence by isolation PA with 2,4-dinitrophenyl hydrazine (DNPH) from an non-heated macerate and demonstrated that it arises enzymatically from precursors.

Relationship between PA and flavor perception for onion pungency determination has been studied by many researchers. Schwimmer and

Guadagni (1962) found a highly significant correlation (r = 0.97) between the amount of enzymatically developed PA in the onion juice and an olfactory threshold concentration of the juice. Wall and Corgan (1992) found that PA values were significantly correlated with mean sensory ratings. Correlation coefficients (r) were 0.92, 0.84, 0.95, and 0.79 in four separate experiments. The high correlation again indicates that PA analysis can be used as a reliable selection technique for pungency in onion-breeding programs.

Schwimmer and Weston (1961) (SW) developed a relatively simple but still somewhat time consuming method to determine the amount of PA. It has become a classical procedure for pungency evaluation. They also reported that onion tissues release PA quickly after the comminution process. Within a period of six minutes after destruction of the cells, 95% of the maximum level of PA developed.

Because of the stability and the analyzability of PA in the plant tissues, many onion researchers use the SW method of PA analysis to measure onion pungency. Some modifications on the procedure have been attempted to reduce the analysis time. Randle and Bussard (1993) reported a new streamlined pungency analysis that reduced the time of sample preparation from 74 minutes to 19 minutes. The key technical innovation in this system was a specifically designed press that extracted onion juice from onion bulbs without solid particles. Yoo *et al.,* (1995) also reported a simplified PA procedure using

undiluted homogenate. They established a highly significant correlation (r^2 0.988) between the new method and the SW method.

Thiosulfinate Determination for Estimate of Pungency

An alternative method for the evaluation of pungency in onions involve the determination of thiosulfinates (Carson and Wong, 1959; Nakata *et a* 1970). The procedure involves derivatizing the thiosulfinates with N ethylmaleimide and measuring the absorbency at the conjugate of 515 nn Freeman and Whenham (1976) noted that other nonthiosulfinates also may giv a positive reaction and interfere with the detection by adding values of detectabl non-thiosulfinates to the result.

Factors Affecting Onion Flavor

Many factors are involved in the determination of onion flavor. Generally there are genetic factors existing in the onion, environmental factors occurrin during onion production and storage, and soil conditions including soil type, sc S content and soil moisture conditions.

1. Genetic factors of onion flavor

The genotype of onion cultivars is one of the most influential facto affecting onion flavor intensity. Lancaster (1984) found that the proportion o flavor precursor varies among cultivars. Flavor levels of over 50 cultivars o onions have been studied and summarized by Lancaster and Boland (1989

Some of the more pungent onions are high-dry-matter cultivars. Most of the mild onions are the Early Grano-type and Japanese cultivars. There was a ten-fold difference in the range of flavor levels among these onions.

The genetic basis of pungency and sweetness of onions has been studied by several researchers. Warid (1952) estimated that the heritability for onion pungency was 71% from parent-offspring regression. McCollum (1968) concluded the heritability of soluble solids in several onion populations to be 60% to 80%. Owen (1961), based upon several segregation populations, reported that there are several genes involved in onion pungency. Lin *et al.,* (1995) and Simon (1995) concluded that additive gene action was more important than dominance for both pungency and soluble solids, based upon their diallel experiment results. Generation means obtained from three crosses made by Lin *et al.,* (1995) indicated an acceptable fit with a simple additive-dominance model, and heritability of 40% for pyruvate and 80% for soluble solids.

2. Soil, nutrition, and environmental factors

Soil and other ecological factors influence the pungency of onion. Platenius (1941) demonstrated that onions grown on peat soils were more pungent than those grown on sandy soils whereas those grown on loam or sandy loam soils were intermediate in pungency. S deficiency in the culture medium of onion plants grown in greenhouse was found to produce very low-

pungency bulbs (Freeman and Mossadeghi, 1973). Randle and Bussard (1993) found that S levels interacted with cultivars in influencing bulb pungency and individual sugars, except for fructose. Freeman and Mossadeghi (1973) also suggested that the water content of the soil in which onions are grown has a marked influence on onion flavor. Their study on S nutrition indicated that an abundant supply of water in the development stage tends to result in large bulbs with relatively low pungency.

Onion flavor is influenced strongly by high temperatures and water supply during the growing season, especially in summer. Freeman and Mossadeghi (1973) found that under dry conditions, onions tend to have higher volatile S content and increased pungency levels.

3. Environment and cultivar interaction

Randle (1992) reported that location and cultivar difference exerted a significant effect on SS and PA in short-day onions in a comparison of green-house grown onions with field-grown onions. However, the interaction of location and cultivar was not significant for these two traits in short-day onions. This interaction was found to be effective only in regard to S content in the onions they studied.

Relationship between Soluble Solids and Pyruvic Acids

Platenius (1944) first reported that total solids content reflects pungency to some extent. Schwimmer and Guadagni (1962) found that there is a modest

correlation (r = 0.57) between SS and PA. Lin *et al.,* (1995) confirmed the existence of a significant correlation between the two traits in an 8-parent diallel experiment. Simon (1995) reported that pungency and soluble solids are correlated among parental inbreds and hybrids but not within F_3s.

Flavor and storability

It has been reported repeatedly that onion flavor may determine onion storability to some extent. Earlier investigators (Owen, 1950; Schwimmer and Guadagni,1962) reported that pungent cultivars were more resistant to neck - rot disease, and they indicated that pungency and soluble solids were positively correlated. Foskett and Peterson (1949) found that mild flavor, poor keeping quality and low dry matter were associated. Recent studies by Lin *et al.,* (1995) found a negative correlation between PA and a neck- rot disease index. They also pointed out that this correlation between pungency and disease was not high in strength and varied among cultivars.

MATERIALS AND METHODS

Plant Materials

Bulb Production

The onion bulbs were produced each year in the field at the Muck Farm of the Crop and Soil Sciences Department, Michigan State University, Lanigsburg, Michigan. Bulbs for sugar and pungency analysis was produced each year from 1991 to 1995. Seeds of the onions were planted in May of each year in field blocks. In each block, the seeds were sown in plot rows. Each plot was 10 m long and 1.3 m wide and had three rows. The plant density was about 225,000 seeds per acre, a typical density used in commercial onion production in Michigan. Two guard rows at the edges of each block were planted using onion hybrids. Each row was sown with 2 g of seed by a hand-operated seed planter. In 1991, 1992 and 1993, Lorsban, a pesticide, was used during the seed planting to control onion maggot.

The soil type of the Muck Farm is a dark-brown peat. Fertilizer, irrigation and herbicide sprays were applied as needed. Field-roguing was conducted to eliminate suspect plants other than the planted genotype based on plant morphology.

Onions were harvested in late September or early October each year. All the bulbs were packed and labeled in onion sacks and transported for storage at

the Horticulture Teaching and Research Center (HTRC), MSU. Freshly harvested onions were allowed to remain at room temperature for curing for about fifty days prior to storage at 0-2 C. The onions were stored until planting in April of the following year.

Seed Production

From 1991 to 1995, selected onion bulbs with known SS and PA were planted along with non-selected bulbs as the control in a sandy field at HTRC in early April of each year. Three types of isolation cages including small cages, medium cages and large tent cages were used to provide the environment for pollination control. Two male and two female bulbs or just four male bulbs were placed together in each small cages (35.5 x 50 cm) which had cloth tubes attached on the bottom. For production of somewhat large amounts of seed, cages of 1 x 2 m (medium sized) were used. Each of the medium sized cages enclosed a small plot of three to four rows. The pollination row was planted in the center of the plot with the seed parents on both sides for crosses. Each row had about ten plants. For a large quantity of seed, onion bulbs were planted in large cages (2.8 x 6.7 m) with 1 or 2 male rows and 4 or 5 female rows (40 bulbs/row) for crosses or six rows of male plants for increase of line populations.

Irrigation and herbicides were applied as needed after planting. The insect-proof isolation cages were set above each of the plots before flowering. Flies were introduced weekly into the small and medium cages and bee hives

were placed in the large cages for pollination. The time frame was usually the last week of June through the third week of July. Roguing was conducted during plant development and flowering to eliminate suspect off-type plants.

Seed harvesting was performed whenever the seed heads were mature usually the last week of August. The harvested seed heads were placed in a drier at about 32-35 C for about 24 hours. After cleaning, the seeds were stored in a 4 C/40% RH-seed storage room.

Onion Germplasm for Improvement of SS and PA

The germplasm chosen for this study generally consisted of the inbred lines which either have been commercially used for hybrid production or have potentials for new cultivar development. The inbred lines, 5718, 8155 and 826 used in this experiment were the parents of the cultivar, Sweet Sandwich. MSU inbreds, 9885 B, 9161 and K B were chosen for this study because they have shown values as possible parents of new hybrids in experimental cases. The cultivar, Sweet Sandwich, is considered to be a low pungent, storable onion. Other commercial cultivars were used as comparison material as necessary and depending on availability at the time of need.

Pollination

1. Self pollination

The onion bulbs of fertile plants were planted in pollination control cages. Seeds from these plants were sown the following year for bulbs of the S1 generation. Selection of the S1 bulbs was made again for SS and PA levels in the spring of the third year. These bulbs were grown again in isolation. Four bulb groups were allowed to mass pollinate in the summer. Seeds from the S1 plants were used to produce S2 bulbs in the fourth year.

2. Intraline crosses between sterile and fertile plants

Crossing between sterile (A line) plants and fertile (B line) plants within an inbred line was performed by planting the selected bulbs of the A and B line plants together in one isolation cage using bees or flies for pollination. Seeds harvested from the A line plants were used to produce the male sterile bulbs of the backcross (BC_0) generation the following year. The following diagram is an example of how a new inbred 5718 A was produced.

Two plants of 5718 A line (high in SS or low in PA) X Two plants of 5718 B line (high in SS or low in PA)

↓

5718 A-HS-LPA (HS and LPA stand for high SS and low PA, respectively)

3. Interline crosses between fertile plants for development of new B lines

A desired trait from a fertile onion plant can be introduced into another line by interline crossing followed by backcrossing. The B line development for an onion inbred usually is carried out by crossing a selected B line with desired characteristics to another B line from which a new fertile inbred line can be developed. The general procedures are diagramed as the following.

Parent 1 (P1) X Parent 2 (P2)
B line plant with a new trait B line plant with the trait to be
(high SS or low PA) improved (low SS or high PA)
↓
Backcrossing 0 (BC_0) generation X P2

↓ Backcrossing with P2 for 5
↓ generations (BC_1...BC_5)
↓ with selection for high SS or Low PA
New B line

In this study, selected bulbs of known SS and PA from two different B lines were planted under a controlled pollination environment in a greenhouse at MSU. The umbels of the plants to be crossed were bagged when the first flower opened. Plants destined to be pollen parents were allowed to flower. Emasculation of the B line seed parents was performed in the early morning and early afternoon every day for a period of 2-3 weeks during flowering. The

anthers of the seed parent flowers were removed by hand before the flowers opened. This was performed by using forceps sterilized with 75% alcohol to avoid possible contamination. Pollen from the designated male plants was transferred with a brush to the stigmas of the seed parent plants. The brush was cleaned in a 75% alcohol solution and dried before the next use.

4. Backcrosses for development of new A lines or B lines with high SS and low PA

Selected bulbs from a two-way crossed sterile line or a three-way crossed sterile line were grown with one of the respective parent B lines (fertile) in the small isolation cages for a backcross generation. Seed harvested from the A line plants were sown the second year to produce the first backcross generation (BC1). The BC1 bulbs were selected again for PA and SS and used to make the second backcross seed parents for BC2 generation. The following diagram shows an example of producing two new sterile lines, 5718 A and 826 B, from a two-way crossed sterile line, 5718 A X 826 B.

Backcrossing		Backcrossing	
(5718 A X 826 B) high PA SS or low PA	X 5718 B high SS or low PA	(5718 A X 826 B) high SS or low PA	X 826 B high SS or low
↓		↓	
5718 A-HS-LPA		826 B-HS-LPA	

5. Two-way crosses and three-way crosses for hybrid seed parents

The seed for two-way cross parent combinations was obtained from the sterile plants (A line) of inbred populations that had been selected and grown with a pollen parent (B line) population of another inbred line in isolation.

The F1 bulbs derived from a two-way cross combination were selected for SS and PA, and planted with the selected bulbs of another inbred line in isolation, to make a three-way cross combination. Seeds from the A line (two-way cross plants) were sown the following year to produce the three-way hybrid onion bulbs. This three-way crossing system is the same as used for the production of commercial hybrids (Pike, 1986).

Two-way cross (sterile) x inbred (fertile)

↓

three-way hybrid (sterile)

Laboratory Analysis

Onion Bulb Sampling

For parental bulb selection for sugars and pungency, the bulbs to be tested were cut into halves. The bottom half of each bulb was saved and labeled for later planting in the field. Only the top half was used for laboratory testing. After cutting, the bottom half cut surface was put into white sand to form a

protective cover. This layer of sand on the onion cut was used to help seal the cut surface, and to reduce moisture loss and pathogen development. Then, the bottom half of the bulb was stored in a refrigerator at -2 C before planting.

To determine the mean pungency and sugar levels in a particular genotype, a random sample of five to eight bulbs was used for a PA test and of six to ten bulbs for sugar analysis.

To determine the transverse distribution of sugars and PA in onion scale layers, scale pieces were cut from two outermost scales, from the two center scales, and from two middle scales. To determine the longitudinal distribution of sugars and PA, slices were taken from the top, the middle and the base of the bulbs.

SS Test

To estimate the total sugar level in a bulb, onion juice from the bulb was used to measure SS. The sample was taken from the inner and middle scales of the bulb. The tissues were sliced and placed into a garlic press, and drops of the onion juice were collected in a 25-ml glass beaker.

Refractometer readings of percent SS were taken individually on each of the bulb samples by applying 1-2 drops of the collected juice on the platform of the refractometer. The SS of each bulb was read under artificial light. Each sample was measured twice, and if the two readings exceeded $\pm$3%, the sample

was checked again. The platform and the glass cover of the refractormeter was washed with distilled water and wiped-dry after each test in preparation for the next sample.

Determination of Sugar Concentration

Results from the colorimetric chemical analysis of sugars may reflect the true level of sugars in the plant tissues more accurately than the refractometer result because it excludes the interference of non-sugar SS which may account for up to 10% of the total SS. To determine the total sugar content of onion bulbs, the colorimetric method (Hodge and Hofreiter, 1962) was applied and modified in this study. Details of the modification and procedure are given later in this section.

The colorimetric method is based on the reaction in which phenol in the presence of sulfuric acid will react with the carbohydrates that have a reducing group (-CHOH) on their molecular structures. This reaction leads to the formation of color products which make it possible for quantitative colorimetric determination of sugars. The method is simple, rapid and sensitive. The color produced is stable.

A wedge of the bulb was cut off longitudinally after the outer, dry scales were removed. A 20-g sample of tissue was diced into cubes (1 cm^3), and blended with 50 ml 80% ETOH in a test tube. The tube was placed immediately in a hot water bath at a temperature of 80 C for 10 min. The extraction tube then

was placed in ice to cool the sample and then was centrifuged at 1.8 k Kg RCF for 6 min. The pellets were extracted with another 50 ml 80% ETOH. After well mixed, the solution in the tube was allowed to stand for 15 min then centrifuged again. One ml of the supernatant then was diluted with 199 ml of double-distilled water. Two ml of the dilution was pipetted into a colorimetric tube and 0.05 ml of 80% phenol was added, then 5 ml of concentrated sulfuric acid was added rapidly. The stream of acid was directed against the liquid surface rather than against the side of the tube to obtain good mixing. The tube was allowed to stand for 10 min in a water bath at 30 C. The absorbency of yellow-orange color of the sample was measured at 490 nm. The sugar content was determined in reference to a standard curve constructed from a series of glucose concentrations (0, 0.5, 1.0, ... 5 mg/ml).

All solutions were prepared in duplicate, except the standard solutions which were in triplicate to minimize errors. Sugar content was calculated and recorded in percentage (%) of the bulb fresh tissue weight (g sugar /100 g fresh tissue weight).

HPLC Analysis for Individual Sugars

In 1993 and 1994, onion sugars were extracted and analyzed by HPLC. Cold-stored onion bulbs were cut into slices and 20 g of samples from each bulb were placed in a styrofoam container. Liquid nitrogen was poured immediately into the container to freeze the tissues for about 2 min. A lyophilizing dryer then

was used to dry the samples for 92 h. The dried onion slices were ground and forced through a 25-mesh screen. Then 100 mg from each sample was extracted with 3.5 ml 80% ETOH. Then each sample was vortexed for about 15 sec and allowed to stand for 15 min, and then, were centrifuged 5 min at a setting of 3000 rpm (=1879 kg RCF) on a Sorvall RT 6000 B tabletop centrifuge. The pellets were extracted two more times using the same procedures as above. The final sugar extract (10.5ml) in each tube was diluted with 5 ml of double-distilled H_2O. In some samples, if any chlorophyll content that may give green color in the tissue sample was suspected, 5 ml of chloroform was added to the tube containing the diluted extract followed by shaking and centrifuging the tube to remove the chlorophyll.

The supernatant, which contained the extracted sugars, was dried thoroughly in a Speed Vac AC 200. Then, 1 ml double-distilled water was added to dissolve the dried sugars. Before being injected into a HPLC analyzer, 1 ml of dissolved dry sugars was diluted with double distilled water to 1: 200 by volume, and 20 ul from the dilution was used for the injection.

The HPLC used was a Dionex Series 4000 I, equipped with a carbopak PA 1 Column (4x250 mm). Each sample was injected with a mixture of 65% water and 35% 200 mM NaOH at a flow rate of 1.0 ml/min. From 0.1 min to 1.5 min the column was eluted with 75% water, followed by 25% 200mM NaOH, and then eluted for 7.5 min with 200 mM NaOH. The carbohydrates then were detected by pulsed amperometry. Standardized 20 PPM of glucose, sucrose,

and fructose were injected before and after three injections of test samples. Each sample was run twice to minimize error.

Pungency Analysis

1. Schwimmer and Weston (1962) pyruvic acid analysis

Onion pungency was estimated chemically by using the SW method of PA analysis. Some modifications were made in this study to fit the requirement of our breeding program. A comparison was made between the result of SW method and the results of the new MSU-developed PA procedure. Onions from same sources were tested for PA levels separately with the two systems in this study.

In this study, the dry outer scales of the bulb were removed, and the bulb was cut into small cubes. Tissue samples of 50 g were chopped into slices. The 50 g samples were placed in a blender with 100 ml of distilled water and blended for 40-60 sec, until no large chunky tissues were visible. After blending, the samples were allowed to stand for 10 minutes to allow development of the PA. Then 10 ml of the onion juice was placed in a 25-ml centrifuge tube. The centrifuge was run at 1.8 k kg RCF for 5 min. Then 1 ml of the supernatant was removed carefully and diluted with 4 ml of distilled water.

Background reactive carbonyls of the samples were checked by adding about 100 ml of trichloracetic acid (25%) to 50 g bulb tissue before blending or

heat application, using a microwave on a maximum heating level (1.5 kw) for 5 min. These treatments were to deactivate alliianase so that enzymatically developed PA would not appear in these treated samples.

After blending and centrifuging, the samples were taken from the prepared supernatant dilution. One ml of the dilution from each sample was placed into a test tube (50 ml capacity). One ml of 0.06% DNPH in 2 N HCL and 1 ml of distilled water then were added to the tube. The tubes were seated in a water bath with a shaking device at 37 C for 10 min. Five ml of 0.6 N NaOH then was pipetted into each sample tube and shaken for 3-5 sec. After the purple color appeared in the tubes, the samples were measured with a spectrophotometer with absorbency at 420 nm, using a reagent blank (2 ml water + 1 ml DNPH + 5 ml NaOH) set at 0 absorbency.

The calibration curve was obtained using sodium pyruvate as the standard. All samples were duplicated. Sodium pyruvate solutions were triplicated.

This procedure was used to determine the section of the bulb best suited for PA sampling (Table 1), to measure the average PA level of the onion lines, and to compare the results obtained with the new MSU-developed pungency procedure.

Development of a New Pungency Procedure for Mass Screening of Onion Populations

2. Pungency determination procedure

The SW PA analysis (1962) requires homogenization of the tissues and spectrophotometric measurement for PA in the final extracts. This method involves substantial amounts of labor and equipment, and restricts the analysis to expensive equipment and well trained personnel. It also destroys the sample bulbs so that the selection for individuals becomes impossible. A new (MSU) procedure was developed in the fall of 1995 which makes PA analysis much simpler. This procedure was designed to be suitable for breeding programs in which mass screening of populations for pungency is desired by breeders, using simple equipment.

In this new procedure, the extraction of onion PA is performed by soaking onion slices with distilled water in petri dishes. A test sample from each petri dish is added to a depression on a porcelain plate and later treated with 2,4-dinitrophenyl hydrazine(DNPH). Then after coloration of the PA-DNPH derivatives, the samples are scored visually according to color intensity. The color intensity of the samples reflected the PA levels in the tissues. Samples high in PA result in a dark purple color, and samples low in PA give a yellow color. Intermediate PA samples show color intensities of light to medium purple.

The MSU procedure was compared with the SW method by analysis of the PA of five onion cultivars, made up of types considered to be sweet or pungent (Fig. 11). Six bulbs from each of five hybrids (Vidalia, Texas 1015, Sweet Sandwich, MSU2518-1 and Duration) were used in this analysis. Vidalia and Texas 1015 were purchased from a local retail store. The other three cultivars were out of the storage from MSU trials. The onion slices were cut horizontally from the middle of the onion bulbs. The size of the slices was about 60 mm in diameter and 5 mm thick. After cutting, the slices were placed in Petri dishes for 10 min to let PA develop. Twenty-five ml of distilled water then was added to each of the dishes to extract PA from the slices. Two drops of the PA sample from each dish were pipetted into a depression on a porcelain plate. Two drops of 2,4-DNPH (0.006% in 2 *N* HCL) then were added to each depression. The plate was kept at room temperature (about 28 C) and gently tipped back and forth a few times during a 12-min time span. Finally, five drops of NaOH (6 %) were added to each sample. Color intensities of samples were estimated visually with four values: Yellow (very low PA) = 1, light purple (low PA) = 2, purple (high PA) = 3, and dark purple (very high PA) = 4.

Samples from the same onion bulbs also were taken and analyzed with the SW method to compare the two methods. The PA concentrations and color values obtained by the two methods were analyzed statistically, using the SAS general linear model (GLM) (SAS Institute, Cary, N.C.).

To check the background PA that was not involved in enzymatic development of pungency, samples of the onion bulbs were wrapped in a plastic bag, and then placed in a microwave oven (1.5 kw) for 5 min to stop enzymatic activity. These samples then were tested for PA by the SW method.

Storage and Onion Flavor Quality

Changes in SS, PA, and Bulb Quality Related to Storage

Onion quality may dramatically decrease due to handling and storage conditions. The bulbs generally can be stored through winter if the temperature and humidity in storage are satisfactory. Onions may respond to storage conditions differently according to their genetic make-up. The flavor quality also changes depending upon genetic and storage environmental factors. To determine how Michigan onions respond to storage time and condition, one experiment was designed and conducted to measure total sugar by the colorimetric method, PA by the SW method, and storability of onion cultivars and breeding material stored from October 1991 to May 1992.

In 1991, six onion samples, K B (line bred cultivar), 826 B and 5718 A (2 MSU breeding lines), 2518-1(an experimental MSU hybrid), 5718 x 8155 (2-way experimental cross) and 'Sweet Sandwich' (hybrid cultivar) were collected from the MSU breeding workshop planting. The onions were harvested in late

September and stored at room temperature (24 C) for two months. After that, the onions were kept in boxes and stored under refrigeration at 0 C from December, 1991 to May, 1992. Each onion line was sampled, with 10 bulbs for total sugar testing and six bulbs for PA analysis during 1, 2, 3, 4 and 5 months' storage. The results are showed in Fig. 3 and 4.

Genetic and Yearly Factors Influencing Onion Flavor

Crosses Among Parental Onions with Different SS and PA

Four cross types involving eight combinations were designed to study how the parental difference in SS and PA influence the flavor strengths of the progeny.

Type 1. high x high: onions high in PA crossed with onions high in PA, or onions high in SS crossed with onions high in SS;

Type 2. low x low: onions low in PA crossed with onions low in PA, or onions low in SS crossed with onions low in SS;

Type 3. high x low: onions high in PA crossed with onions low in PA, or onions high in SS crossed with onions low in SS;

Type 4. low x high: onions low in PA crossed with onions high in PA, or onions low in SS crossed with onions high in SS.

Correlation Between SS, PA and Bulb Sizes

The correlation coefficients between SS and PA, PA and bulb size, and SS and bulb size were calculated from the data from five onion hybrids including 2518-1 a MSU experimental hybrid with a high yielding potential, single bulb

center and globe shape, 'Sweet Sandwich', which generally is considered to be a storable and less pungent hybrid, 'Spartan Banner 80' and 'Norstar' both of which are two cultivars with good storability and high pungency, and 3506 which is another MSU experimental hybrid with a high onion oil content. To obtain bulb size, the diameter of each bulb was measured in cm. Ten bulbs from each entry were used to measure bulb size and test for SS and PA. The SAS general linear model (GLM) (SAS Institute, Cary, N.C.) in Microsoft Window was used to process the data.

Heritability Study

1. Broad-sense heritability

Interline crosses of fertile plants by fertile plants were made in 1991 and 1992. The F_1 plants were selfed in 1993 and 1994. The F_2 bulbs were tested along with the parent onions for PA and SS and SS/PA ratios in 1994 and 1995. Broad-sense heritability (h^2) was calculated by using the variances of the F_2 s and parents. The method was proposed first by Mahmud and Krammer (1951) to estimate broad-sense heritability. SS and PA measurement on F_2 plants of four single-cross populations and on five inbred lines, 5718 B, 8155 B, 826 B, 9161 B, and K B were conducted by taking four bulbs for PA and eight bulbs for SS from each line.

The formula for estimating h^2 was:

$$h^2 = [(\sigma^2_{F2} - \sigma^2_E)/ \sigma^2_{F2}\ ] \times 100$$

where h^2 = broad-sense heritability

σ^2_{F2} = total variance

σ^2_E = environmental variance (non-genetic variance)

Since the environmental variance can be calculated from the variances of the non-segregating populations (P_1, P_2, and F_1):

$\sigma^2_E = (\sigma^2_{P1} x \sigma^2_{P2} x \sigma^2_{F1})^{1/3}$,

σP_1^2 and σP_2^2 = variance of parents of single-cross populations;

therefore, $h^2 = [\sigma^2_{F2} - (\sigma^2_{P1} x \sigma^2_{P2} x \sigma^2_{F1})^{1/3}]/\sigma^2_{F2} x 100$

σ^2_{F1} = variance of F_1

2. Realized heritability

The realized heritability (h_r^2) of SS or PA was determined by the amount of genetic improvement that was realized by selection for high SS bulbs or low PA bulbs within a population.

The formula used was h_r^2 = R/S (Falconer, 1981), where R is the response realized by selection, which is calculated by subtracting the mean of the S1 generation by the mean of parent generation, and S is the selection differential, which is calculated by subtracting the mean of the S1 by the mean of non-selection. The selection differential is the difference between the means of the population from which they were selected.

Five new breeding lines (Table 15) were developed for low or high SS through selfing. Three of the new selfed lines (S_1) derived from two bulbs of

8155 B, 5718 B and 826 B were selected for high SS. Two selfed lines from 8155 B and 9161B were selected for low SS.

Five breeding lines (Table 16) were developed for low PA or high PA through selfing. Three of them (5718 B, 9161 B and 826 B) were selected for low PA, the other two were selected for high PA.

Two to five selected bulbs from each breeding line were planted in one small isolation cage to allow massed pollination. Bulbs produced from the seed of each group were tested again for SS or PA. Realized heritability was calculated using the formula above. The results are shown in Tables 15 and 16.

Frequency Distribution for PA and SS Contents in Parent and Progeny Populations

Crosses between fertile parent plants (P_1 and P_2) of two different families (5718 B x 9885 B and 5718 B x 826 B) provided F_1 seed to produce F_2 populations and B_1 and B_2 backcross populations. Bulbs of these six generations (P_1,P_2, F_1, F_2, B_1 and B_2) were harvested and tested for SS in '5718 B x 9885' B related populations and for PA in '5718 B x 826 B' related populations. The frequency distribution analyses for SS were made based on the data of '5718 B x 9885 B' related populations (Fig. 8). The distribution for PA was made from the data of '5718 B x 826 B' related populations (Fig.9).

The F1 plants of '5718 B x 9885 B' from the seed harvested in 1991 were selfed or backcrossed to the parents in 1993, and bulbs of F2 , the B1 and the B2 were analyzed for SS in 1994. The F1 plants of '5718 B x 826 B' from the seed harvested in 1992 were selfed or backcrossed to the respective parents in 1994. The bulbs harvested in 1995 were analyzed for PA. Relative frequency was calculated by dividing the number of the bulbs with a determined SS value or PA value by the total number of the sampled bulbs, and multiplying by 100.

Tests for SS and PA of Onions for Estimating Yearly Variation

MSU experimental hybrid 2518-1 was used as a standard in SS and PA analyses over five years from 1991 to 1995. Each year, ten and six bulbs from this hybrid were taken for SS tests using a refractometer and PA analysis using the SW method, respectively. Bulbs were tested for SS and PA in March and April each year after winter storage, except the bulbs harvested in 1995 were tested in December 1995. SS:PA ratios were calculated by dividing the mean of SS (%) by the mean of PA (u.ml/g.f.w.). Least significant differences between different years for SS, PA and SS:PA ratio were calculated using t test at $P \leq 0.05$.

Data Analysis

Analysis of variance was conducted to test the progenies after crosses. The crossing combinations without selection for SS and PA were used as the control for the respective progenies. A completely randomized experimental

design was used with two replications in the trials for evaluation of SS, sugar and PA levels in developed onion germplasm. The number of replications for laboratory tests, however, varied depending upon availability of seed and survival of plants in the field. Analysis of variance was performed to test the null hypothesis of no difference in SS percentage, PA concentration and SS/ PA ratio among parent and progeny families. The General Linear Model procedure of SAS in Microsoft for Windows Program was used in regression analysis on pooled data from test results of PA, SS, and bulb size to study the relationship among these traits.

In lab tests for sugar content, SS concentration and PA level of an onion population, 10 bulbs from each population were sampled randomly as ten replications for SS evaluation, six bulbs were used as six replications for sugar and PA analysis. Standard deviations of the mean were calculated to estimate the variability in sugar production and pungency development of each population.

RESULTS AND DISCUSSIONS

Distribution of Soluble Solids and Pyruvic Acid in Onions

The way onion bulbs are sampled affect the final results of flavor analysis. It has been reported that different sections and scale layers of onion bulbs have different levels of flavor components (Bacon, 1957; Darbyshire *et al.*, 1978). Screening of an onion population for SS and PA requires that a part of the onion tissues is homogenized, and the lower base 1/3 of the selected bulbs be saved for seed production. A preliminary study on the distribution of SS and PA in onion bulb tissues was performed in 1991. Information obtained through this study provided the guidance on how to sample onions for flavor tests and helped to explain how the concentration of flavor substances varied among onion tissues.

Changes of SS Contents in Onion Bulbs

To determine the variation in SS among tissues in an onion bulb, five sections of the bulb were sampled, including the outer section (two outer scales), the inner section (from the third to the fifth scale layers), the innermost section (the two center scales of the bulbs), the base section, (the base of the bulbs) and the upper section.

The percentage of SS content of MSU experimental hybrid 2518-1 increased across the scales from the outer older scales to the inner, younger scales. Table 1 shows the means of five bulbs in which the outer tissues contained only 7.64% SS and the innermost had 9.9% SS. Inner tissues were intermediate in SS concentration, with an average of 9.14% SS. Substantial differences in SS contents also were found between the top and bottom sections of the bulbs. The top tissues had a lower SS of 7.9% and the base tissues had a SS mean of 10.12%. Similar results were reported by Darbyshire (1978). He pointed out that older tissues tend to store a smaller amount of SS than younger tissues. Other researchers (Bacon, 1957; Smittle, 1988 and Lin, 1995) reported that the older tissues, including the outer ring and the top of the bulb have large cells. It has been found (Darbyshire, 1978) that large cells are closely associated with low dry weight, sugar and SS, and high fiber content. It is likely that stored sugars are translocated from older tissues to younger tissues of onion bulbs.

Distribution of PA in Onion Bulbs

To obtain information on distribution for PA in onion bulbs, the five different sections of the bulbs were sampled in the same way as for SS tests. The tissues from each sample were blended and tested for PA levels using the SW method.

The data in Table 2 show that PA in onion tissues varies within the bulb. The highest PA concentration was found in the top tissues, which reached 10.92

Table 1. SS (%) determined by the refractive index method and PA ($\mu mol.g^{-1}$) measured by the SW method in onion tissues of MSU experimental hybrid 2518-1.

Tissues [z]	PA	SS
	($\mu mol.g^{-1}$)	(%)
outer	7.64	10.92
inner	9.14	10
innermost	9.9	9.34
regression		
liner	66**	127**
quadratic	0.48^{ns}	0.3^{ns}
F value between groups	8.6**	12.86**
base	10.12	9.12
top	7.9	11.38
F value between groups	7.25*	10.21**

[z] The outer tissues were sampled from the first and second scale layers; the inner tissues were taken from the third to the fifth scales; the innermost tissues were sampled from the center two scale layers of the bulb. The SS and PA contents were the means of five onions.

^{ns},*,** Nonsignificant or significant at $P \leq 0.05$ or 0.1, respectively.

$\mu mol.g^{-1}$. The older and outer onion scale layers also had very high PA at 10 $\mu mol.g^{-1}$. The innermost and the base portions of the scales contained low levels of PA of the tissues sampled. In this study, the PA level increased from the innermost scale tissue to the outer scale tissue and from the base section of the scale to the upper scale tissue. This variation of PA is opposite to that of SS. Lin (1995) reported different results for short-day onions, PA being lower in the second and third scale tissues and higher the outermost (scale 2) and the innermost layers. These SS and PA studies provided the necessary information to design the procedure for selecting bulbs for SS and PA levels, in which, the innermost scales from the center of the bulbs may be used for mean values of SS and PA. There is a significant variation of SS and PA among onion scale layers. The innermost scales and the base tissues of the bulb are statistically different ($P \leq 0.05$) from the outer scales and the top tissues of the bulb in SS and in PA contents.

Variation of SS and PA Contents Due to Storage Conditions

Onion flavor changes in response to postharvest handling and storage conditions. Peterson (1986) reported that the onion cultivar Sweet Sandwich became mild after three months or more in storage. A study was conducted to determine the variation of total sugar and PA content in Michigan onion germplasm in response to storage. These tests also were designed to investigate if there was any particular time that the bulbs could be saved in order to select for desired SS and PA concentrations. Changes in total SS and PA

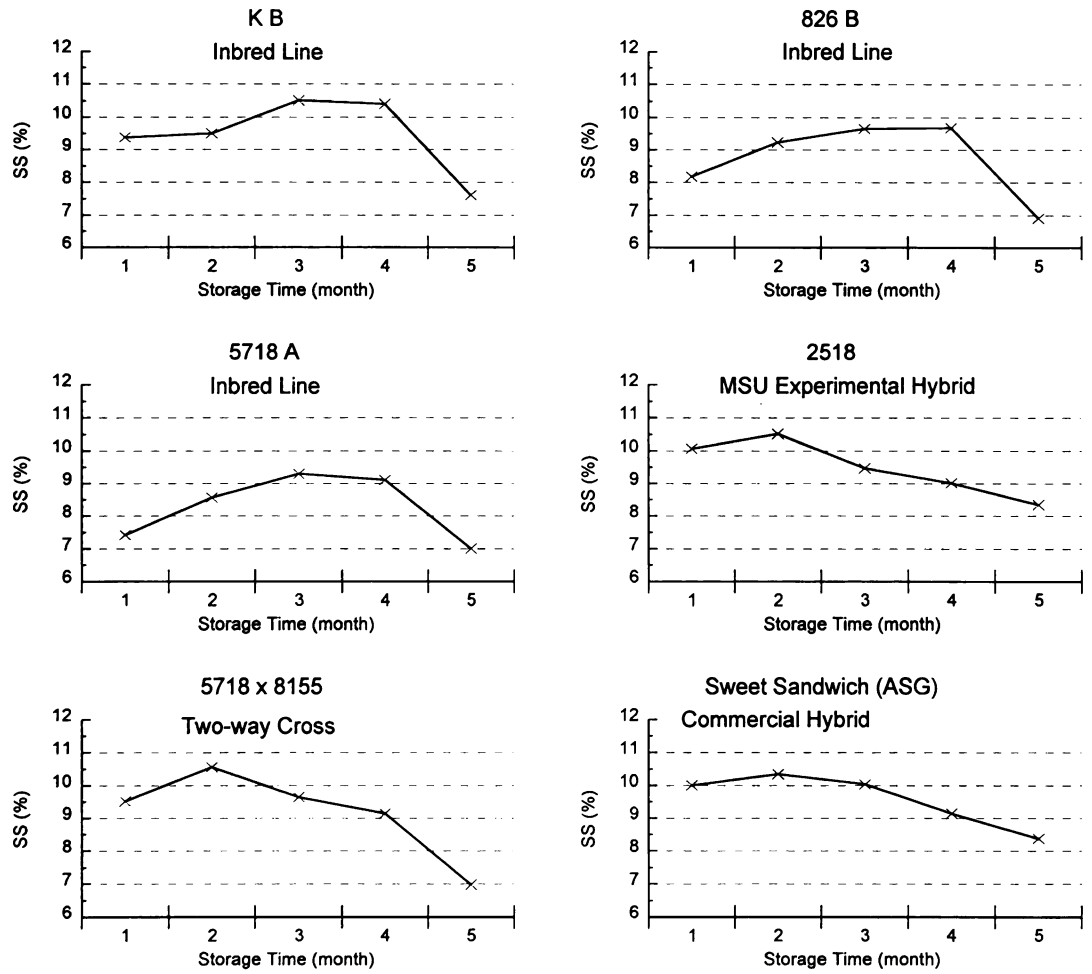

Figure 3. The effect of storage time on SS content (%) in six different onion germplasms. Data points are means of five measurements.

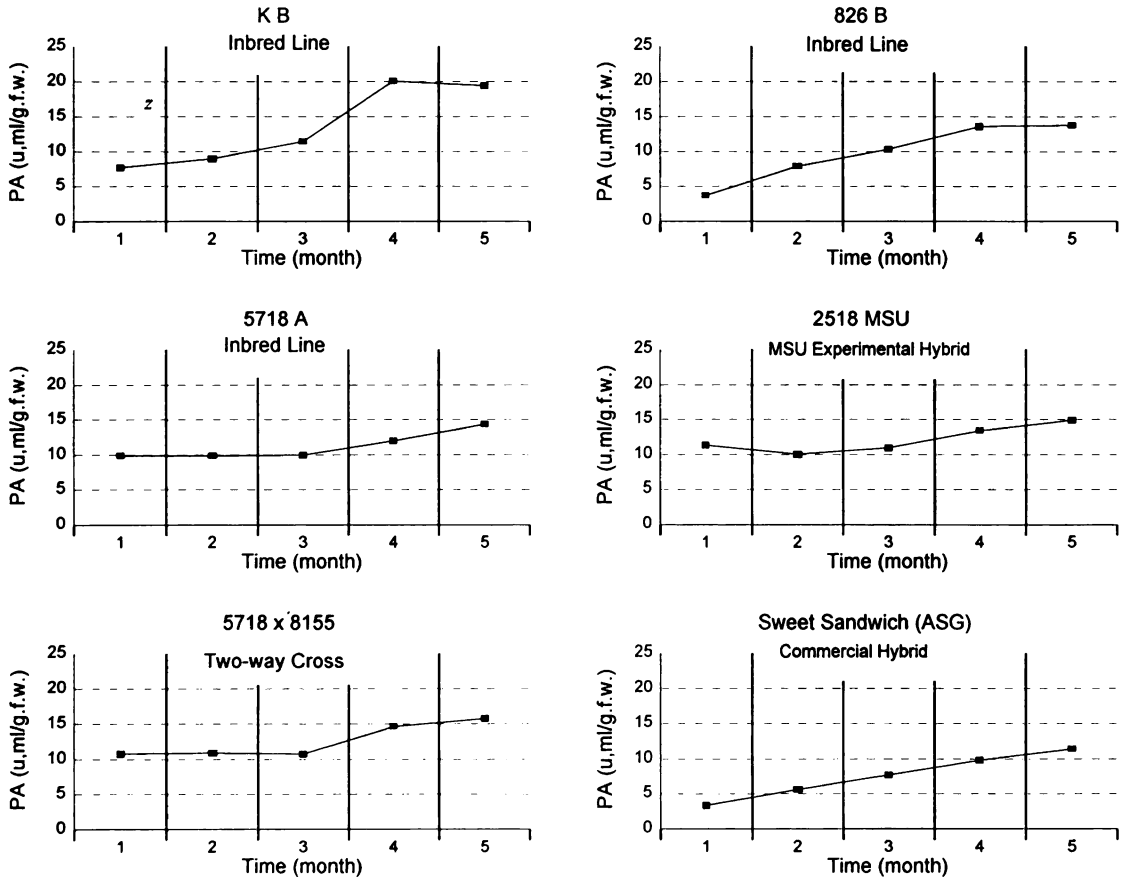

Figure 4. Pyruvic acid (PA) levels over time (5 months) of storage for six different onion germplasms, using the Schwimmer and Weston method. Data points are means of four measurements.

levels in inbreds, K B, 826 B, 5718 A, a two-way cross line 5718 Ax 8155 B, MSU experimental hybrid, 2518-1, and a commercial hybrid, Sweet Sandwich stored at 0 C for 6 months from December of 1991 to May of 1992 are presented in Figures 3 and 4 respectively. Bulbs of onion populations were collected at random each month from January to May. Five bulbs of each onion population were used for SS tests using the refractive index method. Four bulbs from each onion population were collected for PA analysis using the SW method.

Total SS content in the bulbs showed an early increase over the first and sometimes the second month in storage. K B, 826 B, 5718 A increased in SS contents during the first three months and then there was a sharp reduction of SS after four months. After an increase for two months in storage, total SS content in 2518-1, 5718 A x 8155 B and Sweet Sandwich gradually declined. The fluctuation of the SS content in these three hybrids was less than that in the three line-bred onions. Dehydration of the bulbs was observed during storage. This probably is attributable to the sugar increase in the bulbs. Bulbs with higher water content tend to dehydrate more rapidly in storage which would increase sugar concentration. Hybrids with larger bulbs tend to dehydrate slower than the line-bred onions. Therefore, changes of SS content in hybrids should be slower than that in line-bred onions. The sharp drop of SS content at the end of the storage period may be attributable to increased metabolism and translocation of the sugars for energy use in response to shoot development which was observed in most of the line-bred onions.

PA levels in K B and 826 B showed continual increases over five month in storage and 5718 A, 5718 A X 8155 B, and 2518-1 exhibited changes mostly in the last two months of storage. The changes in K B, 826 B, and 5718 X 8155 were significant during the first three months in storage. Again, bulb dehydration in storage probably caused the PA concentration to increase, whereas, increased bulb metabolism after three month of storage (shoot development) would influence PA concentration. Freeman and Whenham (1976) previously reported a similar observation and suggested that during sprouting more precursors are produced and more available to alliinase than before. As a consequence, more PA is produced.

Although SS and PA levels in the onions changed significantly during the storage, the data suggests that after two or three months of storage (February and March), it would be the appropriate time to conduct the selection and evaluation of onion flavor qualities. This is the period of time that the SS and PA levels are the most stabilized in regard to being able to save selected bulbs for planting. This study found that onion bases (lower half of the bulb), if stored more than three months after cutting, became very dehydrated with a high risk for diseases. It is, therefore, recommended that mass screening of onion bulbs from populations for SS and PA be carried out after three months of storage, especially if bulbs are to be saved for planting.

Variation of Sugars and Pyruvates in Onion Germplasm

Variation of SS and PA among Line-bred Populations

Because of severe inbreeding depression, line-bred onions generally are developed by the self-pollination of a single bulb for the development of a fertile B line (or intraline cross pollination for the development of the sterile A line) for two or three generations followed by mass-pollination among a few selected bulbs. A sterile complementary A line is produced by crossing the developed B line to a sterile source and then backcrossing for four or five generations (Pike, 1986). The inbred population remains somewhat heterozygous, which can lead to changes in genetic constitution over time by drift or selection. This level of heterozygosity provides the possibility of genetic variance for the selection of individuals from inbred populations for SS and pungency.

Table 2 shows the SS levels obtained from the test of onion juice refractive index of seven MSU inbred populations. Ten bulbs were sampled for each population. The SS concentration in the bulbs of the inbred lines varied between and within populations. K B had the greatest variation within a population with a standard deviation (STD) of 2.06 and a SS range from 5.1% to 12.3%. The lines 9161 B and 5718 B also had high SS variation within the populations with a STD of 1.56 and 1.49 respectively. The bulbs of 9885 B exhibited relatively less variation in SS, having the lowest STD (0.91) among the populations tested. This

Table 2. Soluble solids[z] (%) in seven onion inbred lines

Inbred	Mean[Y] (%)	*STD*	*C.V.* (%)
5718 A	8.97	1.33	14.83
5718 B	9.98	1.49	14.93
9161 A	9.08	1.08	11.89
9161 B	9.73	1.56	16.03
826 B	9.45	1.14	12.06
K B	9.34	2.06	22.06
9885 B	7.17	0.91	12.69

[z] Determined with refractometer.
[Y] Mean of analyses of ten bulbs

breeding line also had the lowest SS of all the populations with a mean of only 7.17%. Among the seven inbred lines tested, five (5718 B, 9161 A, 9161 B, 826 B and K B) had mean values above 9%.

Although environmental effects on SS of these populations were not estimated, genetic factors still would have to be considered to have a major role in sugar production in the bulbs. The large SS variation in 5718, 9161 and K B may be due to the resident heterozygosity left over during the development of these inbred lines. For instance, 5718 and 826, officially released early in 1982 (Peterson *et al.*, 1986), were originated from self-pollinated single-plant selections in breeding plots at MSU. The selections were mass-pollinated every 2-3 generations to avoid inbreeding depression. A change of genetic constitution over the time would be expected. Such heterozygosity would allow the possibility to select for high or low SS individuals from an inbred population.

PA in MSU Inbred Lines

PA analysis was performed every year from 1991 to 1995 to examine the variation among inbred populations. Tables 4 and 5 show the variation of PA among several MSU inbred lines in 1992 and 1993. PA in onions varied significantly on a yearly basis. The yearly environmental effects on flavor changes are discussed in a later section. In 1993, nine inbred lines were examined for PA, including four populations not analyzed in 1992. The new populations were K2 B, K3 B, 8155 A and 8155 B. The inbred lines K2 B and

Table 3. PA levels[z] in seven MSU onion inbred lines harvested in 1992.

Pedigree	Mean[w] ($\mu mol.g^{-1}$)	*STD*	*C.V. (%)*
K1 B	10.89 a	1.29	11.85
5718 A	9.68 bc	1.04	10.74
5718 B	9.02 cd	0.94	10.42
9161 A	9.43 c	2.12	22.48
9161 B	10.67 b	1.69	15.84
826 B	11.84 a	1.36	11.49
9885 B	10.53 b	2.36	22.41

[z] PA levels were determined with the SW method
[w] Means with the same letter are not significantly different by *T test.*($P \leq 0.05$).

Table 4. PA levels[z] in nine onion inbred lines harvested in 1993.

Pedigree	Mean[w] ($\mu mol.g^{-1}$)		*STD*	*C.V.*(%)
KI B[y]	11.65	a	1.02	8.76%
K2 B	9.21	c	1.98	21.50%
K3 B	7.85	e	2.07	26.37%
5718 B	9.38	bc	1.87	19.94%
5718 A	8.65	cd	0.98	11.33%
9161 A	8.2	d	1.89	23.05%
9161 B	11.45	a	0.89	7.77%
8155 A	8.49	cd	1.23	14.49%
8155 B	7.86	e	1.36	17.30%

[z] PA levels were determined with the SW method.
[y] K1B, K2B and K3B are three different selections within the K B line.
[w] Means with the same letter are not significantly different (*T test*; $P \leq 0.05$).

K3 B are refinement selections out of K1B for single centers and globel shaped bulbs. The inbred line 8155 B is one of the parents for the hybrid Sweet Sandwich.

Tables 3 and 4 show that 826 B, K1B and 9161 B had a very high PA levels more than 10 $\mu mol.g^{-1}$ suggesting high pungency potentials of these line-bred onions. The bulbs of the 5718 B and 9161 A had an average PA value lower than 9.45 $\mu mol.g^{-1}$ as tested in 1992 (Table 3). The bulbs of the 8155 B and K3 B populations were found to be low in PA, less that 7.9 $\mu mol.g^{-1}$ (Table 4), as tested in 1993. The large STD values of the 9885 B and 9161 A populations (Table 3) indicate that these two lines have a large variation for PA within their populations. The highest PA levels were about 40-50% higher than the lowest PA levels in the bulbs within these populations, which should allow selection for very high or low PA individual bulbs. The average means of PA for the inbred lines tested in 1992 and in 1993 (Tables 3 and 4) was different for the two years. A difference in PA was found in the bulbs of K1B which had an average mean of 10.89 $\mu mol.g^{-1}$ in 1992 and 11.65 $\mu mol.g^{-1}$ in 1993. The selection for single center bulbs resulted in populations with reduced PA for K2 B and K3 B, both of which have lower PA than the parent K1 B inbred line. This needs further study to see if there is any relationship between single centers and onion PA levels.

PA and Sugars levels in Storable Onions and Sweet Onions

This analysis was performed in order to understand the difference of total sugar and PA levels between storable onions and sweet onions. A comparison of total sugars and PA for 11 storable onion hybrids and four sweet type onions was made in the summer of 1993 (Figure 5). The storable onion hybrids analyzed were MSU experimental hybrids 3506, 9490, 5534, and four sister hybrids of 2518-1 and cultivars Spartan Banner 80, Sweet Sandwich and Duration. The sweet onions were the cultivars, Vidalia, Sweet Spanish, Walla Walla and Texas Super Sweet. The sugar content in percentage of dry weight was estimated from the total sugars by HPLC analysis. PA was measured with the SW method with modifications. Figure 5 shows the combined sugar and PA levels for these onions. Sugar levels in some of the storable onions are as high as or higher than those were found in the sweet type onions. The four storable onions 9490, 2518-1, 2518-2 and 2518-3) had sugar levels very close to the sweet onion cultivars, Vidalia and TX Super Sweet. The cultivar Sweet Sandwich had a sugar content even higher than Vidalia and TX Super Sweet. Vidalia, however, generally is considered to be a very mild onion, and in this study it had the lowest PA of all. The sweet onions all had a PA concentrations (4-7 $\mu mol.g^{-1}$) lower than the 11 storable onions. The major difference between these storable onions and the sweet onions in regard to flavor is pungency levels. The hybrids Sweet Sandwich and 2518-2 both had a relatively low PA concentration, suggesting the

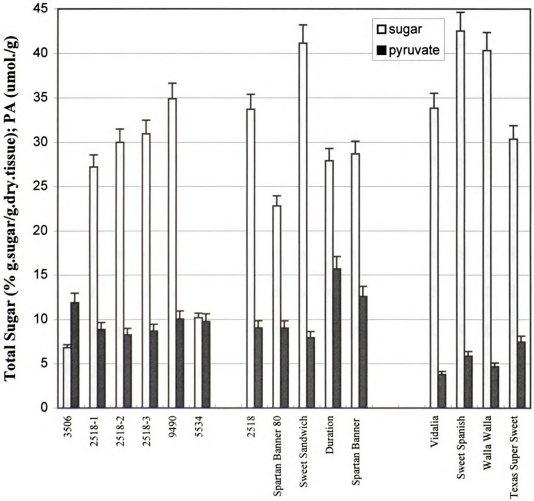

Figure 5. Sugar percentage (g.sugar/g.dry tissue) and PA (μmol/g.) concentration in storable onion hybrids and sweet onions. T bars show the SE for each mean.

possibility for selecting within their parent germplasm populations for storable material with low pungency.

Variation of Reducing sugars in Onion inbred lines and Two-way Cross Populations

Reducing sugars account for a major portion of the total sugars and SS in onion bulbs. Analysis of reducing sugars in onion bulbs would provide useful information for evaluation of the sweetness of onion germplasm or cultivars. This analysis would be more accurate than SS test because the result of reducing sugar analysis excludes non-sugar components which make up a part of the total SS.

Variation in reducing sugar content was found in nine onion populations tested (Table 5). The inbred line 5718 B and two-way cross 5718 x 8155 had the highest reducing sugar levels within the materials analyzed. The three-way cross 5718 x 8155 is the seed parents for 'Sweet Sandwich', a long-day, storable onion bred for low pungency. Evidently, the selection in these two lines during the last five years has made progress toward production for a high sugar onion. Sugar differences between the A and the B line within a breeding line were found, indicating different potential for sugar production in A and B lines. Both breeding lines 5718 B and 9161 B had a higher sugar content than their respective A lines. Continued backcrosses of these B lines with there respect A lines should increase the sugar content in the A lines. The 9885 B breeding line

Table 5. Total reducing sugars in MSU onion breeding lines.

Onion line	Reducing sugars [z] (%)		*STD*	*C.V.*
5718 A	5.95	c [Y]	0.91	15
5718 B	7.8	a	1.08	14
9161 A	5.76	c	0.55	10
9161b	7.1	ab	0.78	11
9885 B	4.68	d	1.13	24
826 B	6.53	bc	0.78	12
K B	7.05	ab	1.28	18
9161 A x 826 B	6.55	bc	0.9	14
5718 A x 8155 B	7.77	a	0.53	7

[z] The Hodge and Hofreiter (1962) method for reducing sugar analysis was used with modifications. A standard curve was established using glucose in a series of concentrations.

[Y] Separation of means in column by least significant difference (*t test*) at $P < 0.5\%$.

had the lowest reducing sugar. This breeding line is one of the parents for an MSU experimental hybrid with high onion oil. Probably, some of the energy products in these plants are converted to oil resulting in less sugar accumulation in the tissues.

Relationship Between Reducing sugars in Onion Tissue and SS in Onion Juice

In order to understand the relationship between the SS and reducing sugars in long-day, storable onions, two inbred lines were used for analysis of SS and reducing sugars. Onion juice was prepared from four bulbs of inbred line 9885 B and 5718 B.

The SS, as measured by a refractive index was related linearly to total reducing sugars in onion juice (Fig. 6) as determined by the Hodge and Hofreiter (1962) method for analysis of reducing sugars in plant tissues with modifications. The data support previous reports by other researchers (Mann and Hoyle, 1945; Bacon, 1957) that the SS and reducing sugar content in onion juice are highly correlated. This study, however, found that the coefficient of determination expressed as r^2, and the Y intercept value that are involved in the description of the relationship between SS and total reducing sugars in onion juice may vary in different onion populations. For the inbred line 9885 B, the equation describing the relationship between SS content and reducing sugars content in the juice accounted for 87 percent ($r^2 = 0.87$) of the variability. For the inbred 5718 B, the

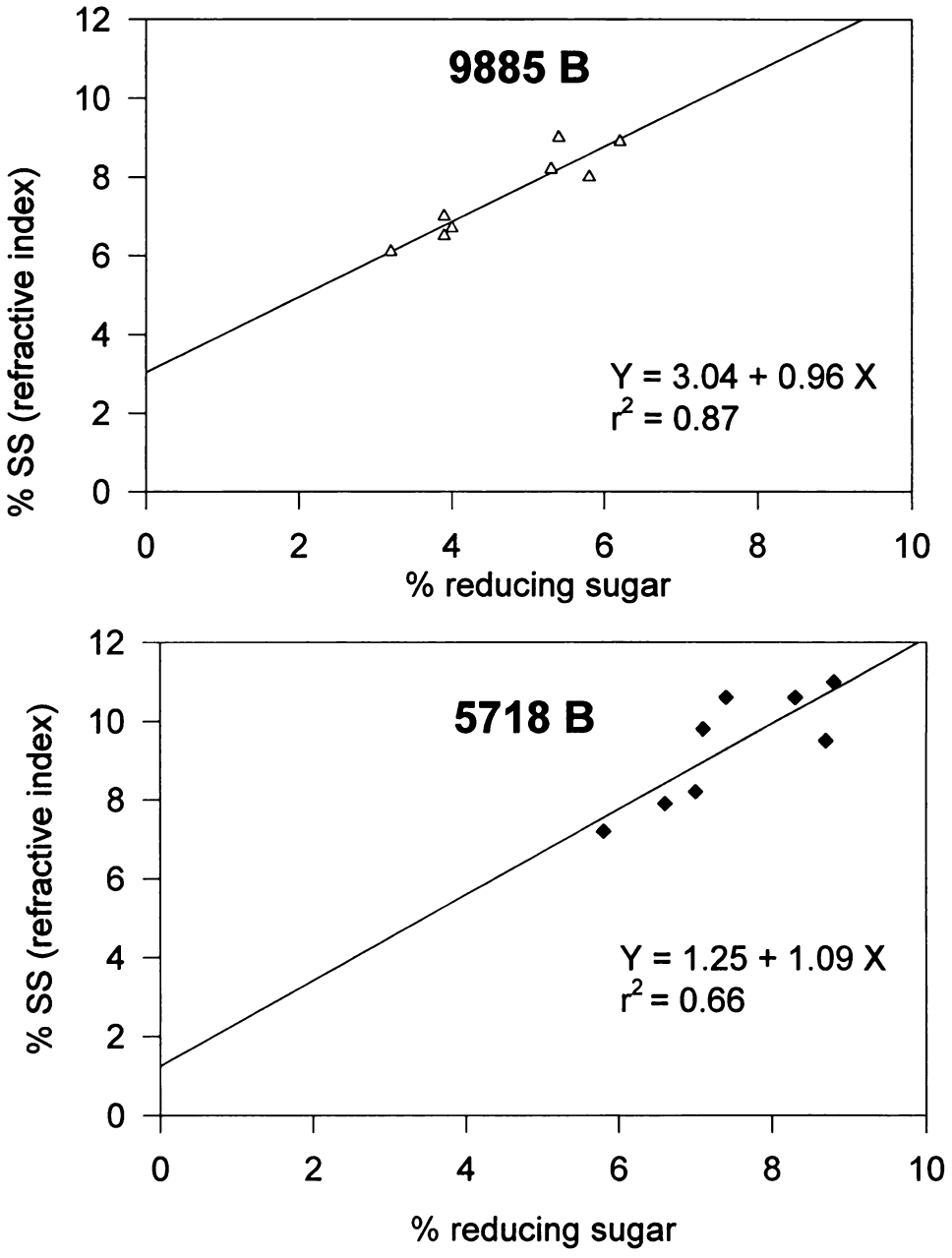

Figure 6. Regression of percent reducing sugars measured by the Hodge and Hofreiter (1962) method on percent SS as measured from the juice refractive index of 9885 B and 5718 B onions.

equation describing the relationship between SS content and reducing sugars concentration in the juice accounted for 66 percent ($r^2 = 0.66$) of the variability. Although, both populations showed a positive correlation between SS and reducing sugars, the inbred line 9885 B had a higher Y intercept value of 3.04 for the regression equation for SS and sugar than the inbred line 5718 B with a Y intercept value of 1.25. These values are useful indicators of the amount of nonsugar solutes present in the juice. For instance, the Y value (3.04) for 9885 B can mean that if the juice is tested at a SS level of 3.04, it, theoretically, contains no sugars but non-sugar soluble solids of 3.04%. In general, 5718 B had a higher sugar content and less nonsugar solutes in the juice than 9885 B.

Results from this study indicate that onion sugar content in the bulb tissue can be estimated by using the SS content as measured by the refractive index of the juice. Although the nonsugar solute present in the juice may interfere somewhat with the estimate of sugar content in different onion populations when a refractormeter is used, it can be ignored since the amount of the difference is small. When a mass screening of individual onion bulbs from a population is necessary, measurement of the SS would be less expensive and less time-consuming than the reducing sugars analysis.

HPLC Analysis of Onion Sugars

Some onions with low total sugar content may taste sweeter than onions with high sugar content. Randle (1993) suggested that it is not only because of

low pungency but also of the different levels of individual sugars. For instance, an onion with one unit of fructose will be tasted sweeter than the onion with 2 units of glucose.

Many techniques can be used to partition onion sugars. One of the most efficient and accurate methods is the application of high pressure liquid chromatography (HPLC). With this method, onion sugars can be separated when the extraction runs through a column equipped in a HPLC instrument and analyzed quantitatively according to the elution time and volume.

Twenty-three onion populations were tested in 1993 with HPLC for individual sugar concentration (% dry weight) in the bulb tissues. The results in Table 6 are from the 'Vidalia' onion, 2518-1, 3506 and 'Sweet Sandwich' populations. Three major sugar components, sucrose, glucose, and fructose were found in the extract injected into the HPLC.

There actually were four peaks being observed on the chromatograms. Before the elution time of the three major peaks, there was a small amount of a sugar molecule appearing on the graph, which had an elution time around 0.5 min. This small peak represents one of the oligosaccharides or fructans in the samples. Fructans are considered to be fructose polymers containing a single glucose residue and commonly found in onion related species (Darbyshire and Hanry, 1978). During storage, fructans can be hydrolyzed to fructose and glucose.

Table 6. Analysis of sugars in onions from three different sources (MSU experimental hybrids, commercial storage onions and sweet non-storage onions) by HPLC.

Onions	Sucrose	Glucose	Fructose	Total
	% dry wt.	% dry wt.	% dry wt.	% dry wt.
MSU hybrid onions				
3506	2.6	2.3	1.9	6.8
2518-1[z]	10.7	11.6	4.9	27.2
2518-2[z]	11.1	12.9	6.0	30.0
2518-3[z]	11.0	14.8	5.2	31.0
9490	12.4	14.8	7.7	34.9
5534	3.1	3.0	4.1	10.2
LSD[y]	*2.07*	*1.89*	*2.2*	*4.01*
Commercial hybrid onions				
2518-1[x]	10.1	11.5	5.1	26.7
S.B. 80	8.6	9.6	4.6	22.6
Sweet Sandwich	18.3	14.5	8.3	41.1
Duration	7.6	14.5	5.8	27.9
Spartan Banner	10.8	8.5	9.4	30.8
LSD	*2.33*	*1.46*	*3.86*	*4.61*
Sweet onions				
Vidalia	13.3	10.3	10.3	33.8
Sweet Spanish	19.0	20.0	3.5	42.5
Walla Walla	17.1	16.2	7.0	40.3
Texas Super Sweet	12.1	12.4	5.8	30.4
LSD	*1.84*	*2.66*	*2*	*2.9*

[z] Different experimental seed production lots of 2518-1.

[y] Data are means of three samples, with lest significant difference (*t test*) at $P \leq 0.05$.

[x] The bulbs of 2518-1 were from a commercial onion trail plot.

Previous researchers (Bacon, 1957; Darbyshire and Hanry, 1978; and Darbyshire, 1978) reported that onions usually had a high content of fructans. In this research the fructan part showed a very small quantity. Possibly, after winter storage of these onions, any oligosaccharides could have been reduced substantially and hydrolyzed in the tissues before the analysis.

Table 6 lists the observed means of sucrose, glucose and fructose of fifteen onions including six MSU experimental line onions, five commercial hybrid onions and four sweet onion cultivars. Total sugar values were calculated by adding the results of the three major sugars. The oligosaccharides or fructans, as noted earlier, are not included because of only trace detection.

The four sweet onions 'Vidalia', 'Sweet Spanish', 'Walla Walla' and 'Texas Super Sweet' all had a high total sugar content, above 30%. The 'Vidalia' onion had the highest fructose level (10.3%), which probably accounts for its sweet taste. The 'Sweet Spanish' onion had the highest sucrose and glucose, but it is not considered as sweet as 'Vidalia'. This may be due to the low level of fructose. Large variation in individual sugars and total sugars were found in the storable, Michigan-produced cultivars. The hybrids 'Sweet Sandwich', 9490 and 2518-1 were high in sucrose, glucose and total sugar, whereas 3506 and 5534 were very low in all three sugar components.

Comparing Michigan-produced onions with the sweet type onion, Vidalia, the major difference is in fructose level. The 'Vidalia' onion generally had a

much higher level of fructose. Other onions in this group all had a relatively low fructose, except for 'Sweet Sandwich' and 'Spartan Banner'. Considering the higher level of fructose in combination with sucrose and glucose and low pungency (Fig. 5), it may explain why the 'Vidalia' onion has much sweeter taste than any other onions. Possibly future breeding work for sweetness improvement of onions should be directed toward selection for high fructose and low PA levels.

Improvement of Sweetness and Pungency in Michigan Onion Germplasm

Intraline Crossing and Backcrossing for Development of High-sugar A Lines

Seed parent line development is essential for new hybrids. Onion inbred A line seeds usually are produced through intraline crossing (A x B). New traits can be introduced into an inbred line through interline crossing followed by backcrossing. To increase the sugar level of seed parental lines, intraline backcrosses were used in this study (Table 7). Total sugar levels of the inbred lines and progenies were determined by the Hodge and Hofreiter (1962) method. Intraline crosses were made between high sugar A lines and high sugar B lines of inbred lines 5718 and 8155. These lines are two of the three parental lines used to produce the cultivar Sweet Sandwich. An interline single-cross also was made between the inbred line of 9161 A and 8155 B, and a backcross of the F1 with 8155 B followed in two years later, using the same selection procedures. The sugar levels in the bulbs of the populations from the crosses were compared

Table 7. Sugar content of onion inbreds and backcross lines following two successive selections of bulbs with and without regard to sugar content[z].

Line	Cross type	Sugar (% fresh weight) in population generations[y]					
		1992			1994		
		BC1			***BC2***		
Backcross (Intraline cross)		mean	Δ^v	*F*	mean	Δ	*F*
5718 A x5718 B	*no sugar determination*[x]	5.40			4.76		
	high x high[w]	7.15	1.75	2.04^{ns}	7.51	2.75	9.78**
9161Ax9161B	*no sugar determination*	5.55			5.23		
	high x high	6.87	1.32	1.76^{ns}	7.22	1.99	4.23*
Single-cross (interline cross)			***F1***				
9161A x8155 B	*no sugar determination*	5.66					
	high x high	6.79	1.23	10.22**			
Backcrossing						***BC1***	
(9 161 A x 8155 B) x 8155 B	*no sugar determination*				6.22		
	high x high				7.54	1.32	5.87*

[z] Hodge and Hofreiter method (1962) for determination of sugars in plant tissues was used.

[y] First selection was done in 1992. Plants derived from first selection are BC1. Second selection was done in 1994, plants derived from second selection are BC2.

[x] Regular bulb selection without regard to sugar level.

[w] Bulb selection with highest sugar level.

[v] Δ is the differential value between the 'high x high' and 'no sugar determination'.

[ns], *,** Not significant, significant ($P < 0.05$)and highly significant ($P \leq 0.01$).

with the sugar levels in the bulbs of the populations without selection.

The test for sugar content in the progenies was first made in 1992. Then reselected bulbs with high sugar levels were mated again. The second test for sugar content in the reselected generation was implemented in 1994. Table 8 shows the results from this selection, comparing bulbs selected without regard to sugar content with bulbs selected for the highest sugar within each population.

The intraline crosses between two high-sugar plants in the families of 5718 and 9161 produced bulbs with sugars higher than their respective selected bulbs without regard to sugar level in 1992. After reselection, the second backcross generation (BC2) of 'high x high' had a higher sugar concentration than the first backcross generation (BC1). If comparing, however, the results between 1992 and 1994, the reselection did not significantly increase the sugars in the BC2 generation, because the yearly effect was high; the bulbs selected without regard to sugar level also increased in sugar at about the same percentage over the 1992 analysis.

The single-cross line, 9161 A x 8155 B also showed an increase in sugar level of 1.23% (the Δ value in Table 8). The 'high x high' progeny of this cross was backcrossed with the high sugar parent line 8155 B. This backcross progeny had a sugar level,1.34% higher than the no sugar determination cross.

Populations of 5718 B and 9161 B inbreds after reselection and selfing of individual bulbs for two generations, exhibited poor vigor, low seed yield, small

bulb size, and poor storability. Further inbreeding (selfing with four bulb masses) probably would reduce the plant vigor seriously with possible elimination of the entire population.

The 'high' selection populations were high in sugars, suggesting a good potential for developing new parent lines with higher sugar levels by selection within the lines. Variation from bulb to bulb in the high x high selection was found for all the sugars measured.

Variation in Sugar Components Determined by HPLC after Selection for High Total Sugar Onions

Sugar content of progeny onions derived from a cross between high SS bulbs of 5718 A and 8155 B was compared to the original parents in 1994. The levels of sucrose, glucose, fructose and total sugars in the bulbs of these three populations were determined by HPLC and are shown in Figure 7. The selection for higher SS not only increased total sugar concentration in the selected progeny population, but the sucrose and glucose levels were higher than either parent. Fructose in the single-cross hybrid population was lower than that of the pollen parent, 8155 B, but the same as in the female parent, 5718 A. These results indicated that although the total sugar content of onions can be improved with a 'high SS x high SS' crossing combination, sugar increase is mainly an increase of sucrose. This also implies that breeding onions for high fructose, which is the sweetest component among the three major onion sugars, may

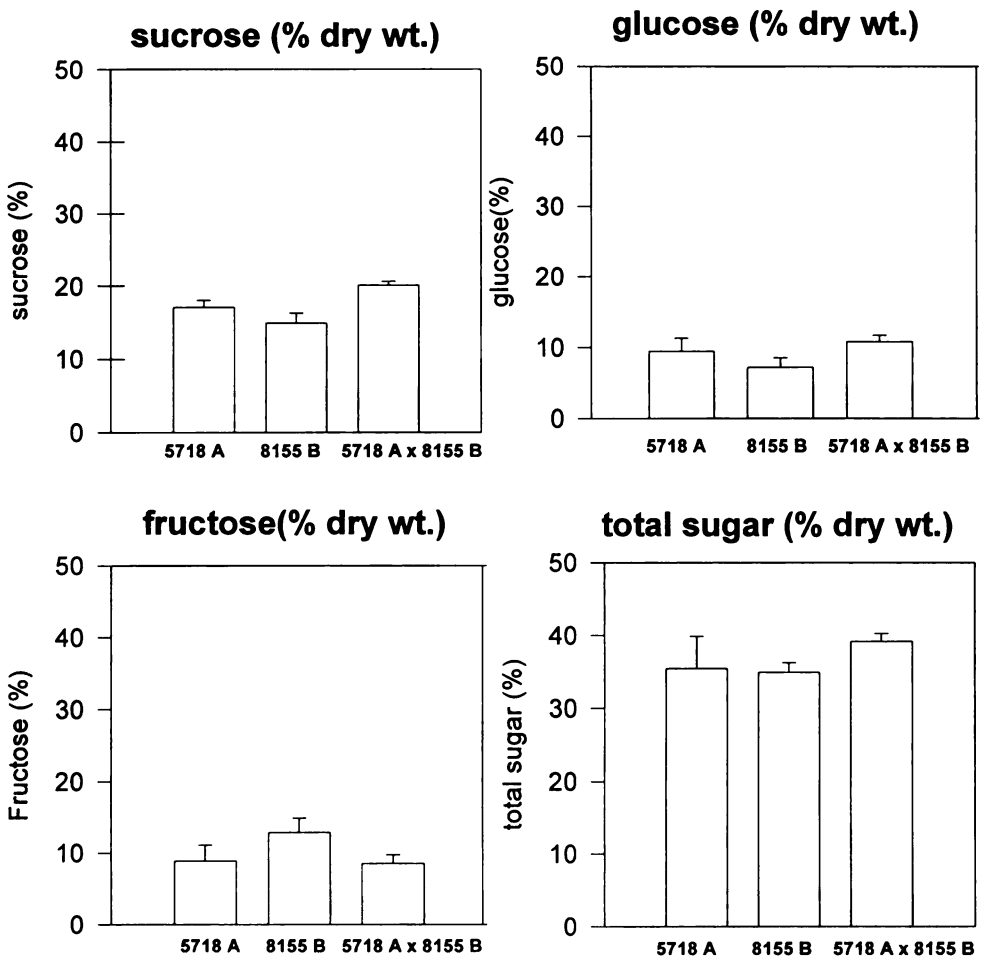

Figure 7. Sugar content of two parental onion lines and hybrid progeny onions derived from high soluble solids bulb selection. Data are means of four bulbs. The mean value of the onion lines as measured with HPLC is represented by a bar, and the error for each bar was calculated at the 95% confidence level.

require a different system, specifically for selection of germplasm high in fructose. Individual bulbs might have to be screened with HPLC which would be time-consuming and costly. Possibly using the quicker, less costly refractometer analysis for SS would indicate total sugars, sucrose and glucose in the selected bulbs, but it would not be an indicator of fructose levels.

Even though the Table 7 results are from reducing sugar analysis and Figure 7 results are from HPLC analysis, the resulting information from both shows that selection for high sugar levels can help increase total sugar in resulting populations. Breeding lines or experimental hybrids might be perceived to be sweeter if the level of reducing sugars could be maintained at 6.5% or higher, or total sugar at 35% or higher, or SS at 11% or higher, and the PA level could be maintained at around 6-7 $\mu mol.g^{-1}$.

Reducing Onion Pungency in Inbreds and Seed Parent Populations

To reduce PA in onion germplasm, intraline crosses, interline crosses, and selfs were used along with low-PA parent bulb selections. Table 8 contains data of PA tests by the SW method in 1992 and 1994 of the progeny populations of four fertile inbreds (B lines) developed by selfing, two sterile inbreds (A line) developed by intraline backrossing, and two single-cross seed parents developed by interline crossing. The selfed populations derived from the low PA selection all showed a lower PA concentration than the controls (regular selection without regard to PA). The difference of PA levels between the two

Table 8. Pyruvate contents determined with the SW method (1961) in onion inbreds and seed parent populations derived from low pyruvic acid (PA) bulb selections.

Line	Cross type	PA (μmol.g^{-1})			
		1992		1994	
Fertile lines		low PA	regular selection [z]	low PA	regular selection
5718 B	selfed	7.92*	8.7	8.46**	10.96
826 B	selfed	9.46 [ns]	9.78	9.25 [ns]	9.9
8155 B	selfed	n/a [y]	n/a	8.02*	9.15
9161 B	selfed	8.33**	10.22	7.22*	10.64
Intraline cross					
9161 A x 9161 B	backcross	6.6**	9.2	8.2**	10.2
5718 A x 5718 B	backcross	7.2*	8.2	7.4*	8.6
Interline cross (two-way cross)					
5718 A x 8155 B	single-cross	5.6**	8.6	7.2*	8.1
9161 A x 826 B	single-cross	7.1**	9.7	6.9**	8.7

[ns],*,**, Not significant or significant by *t test* at $P = 0.05$ or 0.01, respectively.

[z] Regular bulbs selection without regard to PA content.

[y] Data are not available due to missing plots and poor bulb harvests.

types of selection was significant except for the 826 B line.

After a generation of selfing of the inbred line 9161 B for lower PA, this population was more than 30% lower in PA than the regularly selected population (the control). Severe inbreeding depression, however, was observed in the 9161 B population as evidenced by small bulb size and poor vigor. Any further selfing after the second generation, thereforee, would not be advisable.

In the intraline crosses between A and B plants of 5718 and 9161, low PA selection (low x low) produced a progeny of A lines with lower PA in both 1992 and 1994. The difference over regular selection (the control) was significant both years. Significant differences in PA also were found in the interline cross populations indicating that selecting for lower PA was effective, with a potential for the development of less pungent onion hybrids.

Three-way Cross Hybrid Onions with Selection for High SS and Low PA

1. Selection for higher SS

Onion commercial hybrids usually are produced from three-way cross which involved three parental lines. To understand how parental bulb selections for high SS and if any particular three-way cross combinations of germplasm would affect the SS levels in the hybrid populations, two three-way hybrid onions, (9161 A x 826 B) x K B and (5718 A x 8155 B) x 826 B were produced from high-SS parental bulbs. Eight bulbs from each hybrid were produced from the same

Table 9. Soluble solid contents (%) in three-way cross onion hybrids produced from high SS selected parent bulbs or from regular parental bulbs.

Hybrid	Cross type	1992 (%)	1993 (%)	1994 (%)	Mean (%)	Mean gain (%)
(9161 A x 826 B) x K B	*without SS selection*	8.23	8.09	9.46	8.59	
	high x high	9.67	9.88	10.33	9.96	1.37
(5718 A x 8155 B) x 826 B	*without SS selection*	9.75	8.41	8.96	9.04	
	high x high	11.02	10.68	11.14	11.03	1.91

Analysis of Variance for (9161 A x 826 B) x K B

Source	*Sum of Square*	*Degree of Freedom*	*Mean Square*	*F Value*
between groups[z]	2.8	1	2.8	8.22*
within groups	1.36	4	0.34	
total	4.16	5		

Analysis of Variance for (5718 A x 8155 B) x 826 B

Source	*Sum of Square*	*Degree of Freedom*	*Mean Square*	*F Value*
between groups	5.96	1	5.96	20.43**
within groups	1.16	4	0.29	
total	7.12	5		

*, ** Significant at $P < 0.05$ or highly significant at $P = 0.01$, respectively.
[z] Groups are the 'without SS selection' and 'high x high' combinations.

seed lots in 1992, 1993 and 1994 were tested for SS using the refractive index method. The same number of bulbs also were taken from the regular hybrids (no selection for SS in the parent bulbs) of the same seed lots and tested for SS. The results are shown in Table 9. The two hybrids developed from high-SS parent bulb selection are listed as ' high x high'. The regular hybrids are listed as 'without SS selection'.

Three years of testing for SS of the two three-way hybrids, (9161 A x826 B) x K B and (5718 A x 8155 B) x 826 B, showed consistent higher SS levels in the 'high x high' selections. Analysis of variance (ANOVA) results indicate that the selection in (9161 A x826 B) x K B and (5718 A x 8155 B) x 826 B hybrid populations significantly changed the levels of SS based on the three years of data. The hybrid (5718 A x 8155 B) x 826 B had an even higher change in sugar content. The two hybrids from high-SS selected parent materials increased SS level by 1.37% and 1.91%, respectively. This information, again, suggests that selection for high SS parent bulbs can be used to produce three-way hybrids with higher SS levels.

2. Selection for lower pyruvic acid

Selection for lower pyruvic acid parent bulbs for production of the seed of two three-way hybrids, (9161 A x826 B) x K B and (5718 A x 8155 B) x 826 B, was performed for three years (1991,1992 and 1993). Five bulbs from each of these hybrid populations, including the ones from the same seed lots without PA

Table 10. Pyruvic acid (PA) contents ($\mu mol.g^{-1}$) in two three-way cross onion hybrids produced from parent bulbs with or without the selections for low PA levels as determined by the SW method.

Hybrid	Cross type	1992 $\mu mol.g^{-1}$	1993 $\mu mol.g^{-1}$.	1994 $\mu mol.g^{-1}$.	Mean $\mu mol.g^{-1}$.	PA reduction (%)
(9161 A x 826 B) x K B	*without PA selection*	9.2	8.4	10.6	9.4	
	low x low	8.2	5.6	6.8	6.9	-2.5
(5718 A x 8155 B) x 826 B	*without PA selection*	8.2	9.1	7.4	8.2	
	low x low	6.2	7.8	5.5	6.5	-2.7

Analysis of Variance for (9161 A x 826 B) x K B

Source	*Sum of Square*	*Degree of Freedom*	*Mean Square*	*F Value*
between groups[z]	9.6	1	9.6	6.56 **
within groups	5.9	4	1.47	
total	15.49	5		

Analysis of Variance for (5718 A x 8155 B) x 826 B

Source	*Sum of Square*	*Degree of Freedom*	*Mean Square*	*F Value*
between groups	4.5	1	4.51	4.26[ns]
within groups	4.2	4	1.06	
total	8.7	5		

[ns],** Not significant or highly significant at $P = 0.01$, respectively.

[z] Groups are the 'without SS selection' and 'high x high' combinations.

selection, were tested in 1992, 1993, and 1994 for PA contents with the SW method. Progeny from the two 3-way cross hybrids using parental material selected for lower PA levels showed lower PA levels over three years of analysis (Table 10).

The cross of low by low of the hybrid (9161 A x 826 B) x K B , produced a lower mean PA level of 6.9 $\mu mol.g^{-1}$, whereas the non-selected PA control had a higher mean PA of 9.4 $\mu mol.g^{-1}$. The ANOVA showed that the change in the selections (Table 10) was not found to be statistically different from the control. The 2.5 and 2.7 percent reduction, respectively in the PA level in these two 3-way hybrids, however, is within the desired 6-7 range of $\mu mol.g^{-1}$ of PA.

Genetic Analysis of Pungency and SS in Storable Onions

Effects of Selected Parents for SS and PA Levels on Progeny Populations

To obtain an estimate of response to different selection pressures for SS and PA, progeny population following four different crosses (high x high, low x low, high x low and low x high for SS and PA) involving six cross combinations (Tables 11 and 12) were compared to the onions of the same combinations without selection as the control in 1992 and 1994. The two years of data showed that the high x high SS crosses and the low x low PA crosses generally were the most effective for increasing SS and reducing PA in the progeny populations. Significant changes in SS and PA were found in the onions mostly

Table 11. Soluble solids (SS) content of onion progenies derived from parental bulbs selected for different SS levels determined from refractive index of onion juice.

Combinations	SS (%) [z]									
	1992					1994				
	high x high	low x low	high x low	low x high	control	high x high	low x low	high x low	low x high	control
Backcrosses										
5718 A x 5718 B	11.8**	7.2*	7.9[ns]	8.2[ns]	8.2	13.4**	7.4*	8.2[ns]	8.0[ns]	8.6
9161 A x 9161 B	12.8**	6.6**	8.6[ns]	8.8[ns]	9.2	14.5**	8.2**	11.2*	8.0**	10.2
2-way crosses										
5718 A x 8155 B	13.8**	8.0*	8.4[ns]	7.9*	8.6	13.6**	7.6**	9.0[ns]	8.8[ns]	9.4
9161 A x 826 B	10.6*	7.2**	6.8**	9.5[ns]	9.2	14.0**	9.2[ns]	8.8[ns]	9.2[ns]	8.6
3-way crosses										
(5718 A x 8155 B) x 826 B	14.8**	8.0**	8.8[ns]	9.4[ns]	10.6	13.5**	7.2**	7.8*	10.1[ns]	9.8
(9161 A x 826 B) x K B	12.5**	7.9[ns]	8.5[ns]	8.2[ns]	8.4	11.8**	8.2**	8.1**	9.0*	10.2

[ns], *,**, Not significantly or significantly different from the value of control at $P \leq 0.05$ or 0.01, respectively.

[z] Means of 6 progeny bulbs.

Table 12. Pyruvic Acid (PA) levels in onion progenies derived from parental bulbs of different PA levels determined with the SW method.

Combinations	PA levels ($\mu mol.g^{-1}$) [z]									
	1992					1994				
	high x high	low x low	high x low	low x high	control	high x high	low x low	high x low	low x high	control
Inbreding										
5718 A x 5718 B	12.3[ns]	7.8**	10.4**	9.6**	12.1	13.5**	6.8**	10.0*	12.4*	11.3
9161 A x 9161 B	14.6**	8.4[ns]	9.8*	8.7[ns]	8.8	11.0**	6.4**	7.9*	10.5*	9.2
1-way crossing										
5718 A x 8155 B	10.9**	5.6**	6.8**	7.3*	8.6	9.6**	7.2*	10.2**	8.2[ns]	8.1
9161 A x 826 B	16.4**	7.1**	8.6*	8.4*	9.7	14.0**	6.9**	6.8**	9.4[ns]	8.7
2-way crossing										
(5718 A x 8155 B) x 826 B	8.6[ns]	6.2**	7.9[ns]	8.0[ns]	8.2	13.4**	5.5**	8.3*	7.2[ns]	7.4
(9161 A x 826 B) x K-1	18.7**	8.2*	9.9[ns]	10.3[ns]	9.2	16.4**	6.8**	8.7**	8.4**	10.6

[ns], *,**, Not significant or significant at P = 0.05 or 0.01, respectively.

[z] Means of 5 progency bulbs.

from the high x high and the low x low crosses. The high x high SS crosses gained significantly higher SS (mostly at $P \leq 0.01$) than the control and the low x low gave significantly lower SS than the control for ten out of the twelve crosses. The low x low SS cross in the combination of (9161 A x 826 B) x K B, however, was not significant but still lower than the control. The low x low 9161 A x 826 B cross of 1994 for SS was completely different to the 1992 cross. The 1992 cross was highly significant for a lower SS level, whereas, the 1994 level was not significantly different. The progenies of the high x low and the low x high crosses responded differently to the parental selection. Most of these combinations (17 out of 24) for SS levels did not result in a significant change in SS.

These same high x low and low x high combinations for PA levels gave rather varied results. There were eleven values significantly lower than the control and 5 values significantly higher than the control. It is difficult to draw any general conclusions to suggest if any one of these two crosses (high x low ;low x high crosses) would lead to respective increase or a decrease in SS and PA contents in the progeny of such crosses. The results indicate that if higher SS and lower PA levels are desired it should be possible by selection of bulbs that have been analyzed accordingly. This supports suggestions of other researchers (Bacon, 1954; Lin *et al.,* 1995 and Simon, 1995) that SS and PA in onions are traits controlled by additive genes and that additive genes have small individual effects and are influenced markedly by the environment. In the case of low x

high and high x low crosses, possibly any additive influence would be working against itself causing varied results such as observed in this analysis.

Correlation Among PA, SS and Bulb Sizes

When comparing PA to SS, some researchers assumed that there was a linear relationship between SS and PA (McCollum, 1968; Lin *et al.*, 1995; Simon, 1995). In short-day onions, PA and SS also were related linearly to bulb size (Lin *et al.*, 1995). In this study with five storable onion hybrids (MSU experimental hybrids 2518-1 and 3506; commercial onions 'Sweet Sandwich', 'Spartan Banner 80', and 'Norstar'), the correlation coefficients of PA, SS and bulb sizes calculated from the data of these five onion lines were significant, except for PA:SS for MSU experimental 3506 and SS:Size for MSU experimental 2518-1 (Table 13). PA content in four of the five hybrids had a moderately positive correlation with SS, which ranged from 0.52 to 0.61, suggesting that the onions with higher PA tend to contain more SS in the bulb tissues. PA content in 3506 had a low correlation coefficient with SS, which was not significant, indicating another possibility that the relationship between SS and PA may vary according to onion hybrid. Bulb size of all five hybrids had a negative correlation with SS and PA, indicating large bulbs tend to have lower concentrations of SS and PA in their tissues. Because of the positive relationship between SS and PA in some of the onion populations, it may be difficult to breed for an onion with high SS and low PA. Due to this relationship, breeding, therefore, for high SS

and low PA storage onions may requiring more extensive analysis over a longer time frame.

Table 13. Correlation coefficients (r) of pyruvic acid (PA), soluble solids (SS), and bulb sizes in five onion varieties.

Onions	PA:SS	SS:Size	PA:Size
		r	
2518-1[z]	0.59 **	-0.49	-0.69 **
Sweet Sandwich	0.53 *	-0.62 **	-0.53 *
Spartan Banner 80	0.61 **	-0.54 *	-0.64 **
Norstar	0.52 *	-0.66 **	-0.51 *
3506[z]	0.22	-0.56 *	-0.63 **

[z] MSU experimental hybrids.
* Significant at 5% level.
** Significant at 1% level. Each r was calculated in a regression analysis based on the data of 10 onion bulbs from one population.

The negative correlation among bulb size and PA, and SS suggests that large bulbs of storage onions tend to have lower concentrations of SS and PA. It is natural to favor large bulbs for breeding germplasm, because of their yield potential and mild flavor. This relationship, however, may be based upon the moisture content in the bulb. Large bulbs usually contain a larger amount of water that dilutes all the substances in the cells. As large bulbs become drier at the end of a long store period large, mild onions can become more pungent too (Platenius, 1944).

Broad-sense Heritability for SS, PA and Ratio of SS to PA in Onions

To study inheritance of SS and PA, interline crosses were made between fertile onions with high SS and low PA in 1991 and 1992. The F1 onions were selfed in 1993 and 1994. A variance components procedure (Mahmud and Kramer, 1951) was used to calculate the broad sense heritability for SS, PA and SS/PA ratio (Table 14).

Broad-sense heritability for SS, PA and SS/PA ratio for four onion populations was estimated, and the results are presented in Table 14. The values obtained from different lines vary to some extent. Heritability of SS, PA and SS/PA ratio ranged from 87% for 5718 B x 826 B, to 73% for 9161 B x 826 B for SS, 68% for 9161 B x 8155 B to 47% for 5718 B x 826 B for PA, and 77% for 9161 B x 826 B to 59% for 5718 B x 826 B for SS/PA ratio. These values are useful as they give an estimate of maximum heritability for SS, PA and SS/PS

Table 14. Broad-sense heritability (H^2)[z] estimated for soluble solids (SS), pyruvic acid (PA) concentrations and SS/PA ratio for four onion populations.

Crosses	H^2 SS (%)	PA(%)	SS/PA(%)
9161 B x 8155 B[y]	74	68	68
5718 B x K B[y]	84	59	67
5718 B x 826 B[x]	87	47	59
9161 B x 826 B[x]	73	60	77
Mean	84.5	58.5	67.8

[z] H^2 value was calculated using a variance components procedure (Mahmud and Kramer, 1951) based on the data of SS tests by using onion juice refractive index and PA analysis (Schwimmer and Weston, 1961). Eight and four bulbs from each population were used for SS test and for PA analysis, respectively.

[y] Data of 1994.

[x] Data of 1995.

ratio in these populations. For an example, the two interline crosses, 5718 B x 826 B and 5718 B x K B which have the H^2 value more than 84% would have a higher genetic potential to produce high SS progeny than the two other interline crosses, 9161 B x 826 B and 9161 B x 8155 B with a H^2 value less than 75%. In general, these four interline crosses have a higher H^2 value for SS (73-87%) than for PA (47-68%), indicating that selection for SS is generally more effective than for PA.

Realized Heritability in Selfed Inbred Populations

Five reselected inbred fertile lines were developed from selecting progeny from selfed bulbs for either low SS or low PA and for either high SS or high PA. The reselections within the inbred populations of 5718 B, 9161 B and 826 B resulted in changes for SS and PA levels in the S_1 progenies as is shown in Tables 15 and 16. The mean values of S_1 populations from high-SS selection were all higher than their parents and the mean values of S_1 populations from low-SS selections were all lower than their parents. The same trend also was observed in the PA selection, but the changes in PA were smaller than that in SS. The amount of genetic improvement realized by this type of selection was estimated by calculating the realized heritability H_r^2. The highest H_r^2 value for SS was found in the population of 826 B, indicating that reselections in this population is highly effective. The second highest H_r^2 value for SS was found in 8155 B. These two inbreds are the major parental lines of the three-way cross hybrid, 'Sweet Sandwich'. These results indicates that selections for high SS in

Table 15. Realized heritability (H_r^2) for soluble solids (SS) (%) in five inbred onion fertile (B) lines derived from selfing high SS or low SS parental bulbs.

Selfed inbreds	Selection for SS	Mean of parent	Mean of S_1	S [z]	R [x]	H_r^2 [y]
		(%)	(%)			(%)
8155 B	high	10.4	14.6	4.8	4.2	87.5
5718 B	high	10.2	13.6	4.6	3.4	74
826 B	high	8.8	11.2	2.6	2.4	92
8155 B	low	11.4	8.0	-4.9	-3.4	69.4
9161 B	low	8.2	6.5	-2.4	-1.7	70
Mean				3.86	3.02	78.6

[z] Selection differential = mean of S1 - mean of control (without selection).

[y] Realized heritability, H^2 = R/S (Falconer, 1981).

[x] Response realized by selection = mean of S1 - mean of parent.

Table 16. Realized heritability (H_r^2) for pyruvic acid (PA) content ($\mu mol.g^{-1}$) in five inbred onion fertile (B) lines derived from selfing high PA or Low PA parental bulbs.

Selfed inbreds	Selection for PA	Mean of parent	Mean of S_1	S [z]	R [x]	H_r^2 [y]
		($\mu mol.g^{-1}$)	($\mu mol.g^{-1}$)			%
5718 B	low	10.96	8.46	-2.91	-2.5	85
9161 B	low	10.64	7.82	-3.6	-2.82	78
826 B	low	9.9	9.25	-1.89	-0.65	34
5718 B	high	10.96	13.6	3.7	2.64	71
9161 B	high	10.64	12.5	2.3	1.86	81
Mean				2.88	2.1	69.8

[z] Selection differential = mean of S1 - mean of control (without selection).

[x] Response realized by the selection =mean of S1- mean of parent.

[Y] Realized heritability, H^2 = R/S (Falconer, 1981).

these two line population possibly lead to a higher SS level in this hybrid. In Table 16, among the five inbred lines, the response (R) of 9161 B to the selection for PA was the greatest (-2.82), meaning that through the selection the progeny PA mean value has been reduced and is 2.82 ($\mu mol.g^{-1}$) lower than the mean value for the parent. The largest H_r^2 value, however, was found for the selection of 5718 B. H_r^2 values for PA for both 5718 B and 9161 B were large and suggested this type of selection is possible for PA improvement of these two lines. The inbred 826 B line was the only line that failed to show H_r^2 large enough to justify the genetic improvement by selfing and selection for low PA. It could be due to the homozygosity of the parental population, as low PA was already high at the time of selection, which would make the selection among the population less effective. The average H_r^2 value for SS (78.6%) is higher than that for PA (69.8%), indicating selection in these selfed inbred line populations for high or low SS is more efficient than for high or low PA.

Relative Frequency Distributions of Individuals for PA and SS in Two Parent B Lines and the Progeny Populations

Crosses were made by pollinating 5718 B plants containing high SS or low PA levels with 826 B plants containing low SS or high PA levels. Knowing the levels of SS and PA in the parent and progeny populations made it possible to determine the frequency distribution for SS and PA within the F1, F2, and backcrossing Bp1 and Bp2 generations. The frequency distributions for SS and

PA are shown in Figures 8 and 9. The relative frequency (%) was calculated by dividing the number of the bulbs at a SS or a PA level with the total bulb number sampled from the population. In Figures 8 and 9, the parent and progeny populations are represented on the horizontal axis, and mean values of SS and PA are represented on the vertical axis.

These two figures show the typical mode of inheritance for additive gene action controlling the two traits, SS and PA levels. Each of them have a continuous distribution figure on the graphs with the F1s and F2s intermediate between the P1s and P2s, and the Bp1s intermediate between the P1s and F2s, and the Bp2s intermediate between the P2s and F1s.

In Figure 8, there were two separate means of SS for the parental frequency distributions (P1 and P2). P1 had a distribution graph at a mean near 9%, and P2 appeared to have a distribution at a mean near 8%. The F1 distribution was relatively narrow, with a mean positioning from 7 to 11%, similar to the high SS parent, P1. Bp1 was strongly skewed toward P1 while Bp2 was skewed toward P2. The F2 frequency distribution was relatively wide and less symmetrical with a mean positioning from 7 to 10%. This indicates again that there is additive inheritance for SS. The F1 population had more individuals with the gene effect for these two traits. The F2 distributions for both SS and PA were typical and suggestive of additive inheritance. The means of the progeny high SS than the F2 population. There is also the possibility that heterosis in the F1s may have resulted in increased sugar production in the bulbs. In Figure 9,

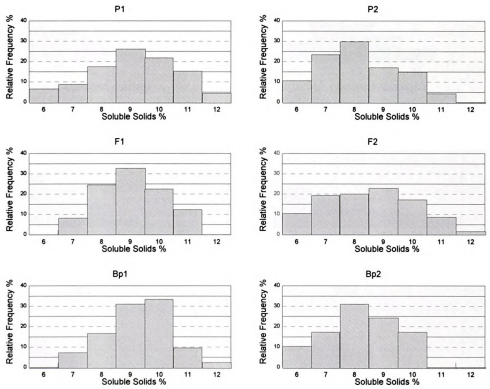

Figure 8. Frequency distribution of percent SS content of the onion parents and progenies of 5718 B x 826 B. The P1 and P2 represent 5718 B and 826 B respectively; the F1 is the progeny of 5718 B x 826 B; the F2 is the progeny from selfing the F1 (5718 B x 826 B); Bp1 is from the backcrossing of the F1 (5718 B x 826 B) with 5718 B; Bp2 is from the backcrossing of the F1(5718 B x 826 B) x 826 B.

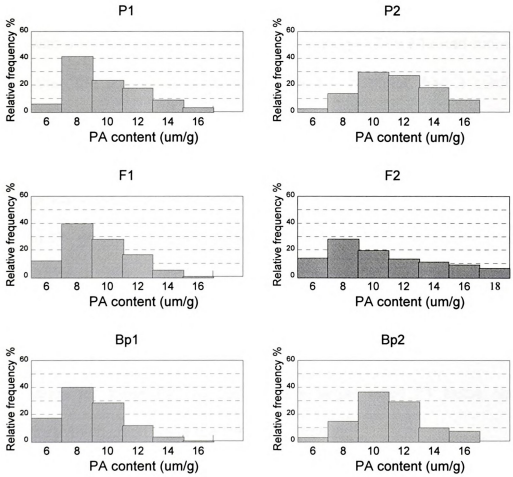

Figure 9. Frequency distribution of PA concentration of the parents and progenies of 5718 B x 826 B. The P1 and P2 represent 5718 B and 826 B, respectively; the F1 is the progeny of 5718 B x 826 B; the F2 is the progeny from selfing the F1 (5718 B x 826 B); Bp1 is from the backcrossing of the F1 (5718 B x 826 B) with 5718 B; Bp2 is from backcrossing of the F1 (5718 B x 826 B) with 826 B.

although the frequency distributions of P1 and P2 covered a range of PA from 8 to 10 $\mu mol.g^{-1}$ while the P2s had two major portions of bulbs (about 55% of the total) with a PA level in a range from 10 to $\mu mol.g^{-1}$. The F1s had more low PA bulbs than the P1s. The distribution of the F1, however, had a configuration similar to the P1, except that high PA (16 $\mu mol.g^{-1}$) bulbs were not found in the F1s but in the P1s. The F2 distribution showed again a relatively wider and less symmetrical distribution than the F1 and exceeded the range of that found in the P1 and P2 graphs. The two backcrosses had distribution configurations very close to their respective parent lines in regard to total spread and peak frequencies. Bp1 had a low frequency of less than 10% in the 14 $\mu mol.g^{-1}$ range and an absence in the 16 $\mu mol.g^{-1}$ range. This could be due to additive dominance for lower PA, or possibly the larger sized F1 bulbs had more moistures which could dilute the PA concentration.

Lower level of PAs and higher level of SS in the F1s than that found in the F2s suggest that herterosis for vigor in the F1 bulb may have increased SS, and the large bulb size of the F1s may have improved the PA trait . In general, the frequency distribution graphs for SS and PA provided useful information about populations (F1s, F2s and BCs) also indicate additive-dominance of high SS over low SS and low PA over high PA. These results of frequency distribution for SS are similar to the early reports by Warid (1952) and Owen (1961). Warid estimated that there are 4-10 gene pairs involved in determination of the SS level in onions after crossing high SS onions with low SS onions. He also

concluded that partial dominance of low SS is involved. Owen, who examined the segregation of SS genes in short-day onions, also suggested an additive-dominance mode in the inheritance of SS. They both used Spanish type onions in their studies. The information from this study indicates that the additive-dominance mode for SS is also suitable for long-day onions. Additive gene effects on PA also were suggested by other researchers (McCollum,1968; Simon, 1995; and Lin *et al.*,1995). They all reported heritability of pungency measured by PA analysis to be fairly high and suggested additive-dominance gene action for low PA over high PA in onions.

Yearly Variation in SS and PA of Onions

Year-to-year variation in SS and PA were evaluated for onion population 2518-1. PA, and SS:PA ratios (Table 17) differed significantly among the five harvest years. The only year in which the onions had an SS content significantly different from other years was 1994. The percentage of SS showed less significant yearly variation, suggesting that SS content is influenced less by environmental variables than PA. Data of 1995 showed lower concentrations for both SS and PA than any other year. This may be due to the fact that the bulbs had higher water content; as they were analyzed after two month in storage which is 2-3 month earlier than when the onions were tested in the other four years. Year-to-year variation in the SS:PA ratio may be explained by the large variation of PA among the test results of these five harvest years. Because SS levels of onion bulbs are influenced less by environmental factors, the yearly

Table 17. Variation in SS, PA and SS:PA ratio in MSU experimental hybrid 2518-1 over five years.

Year	SS	PA	SS:PA
	(%)	(μmol.g^{-1})	
1991	8.6 a[z]	9.2 b	0.93 b
1992	9.23 ab	11.25 c	0.82 a
1993	8.2 a	8.27 ab	0.99 c
1994	9.7 b	10.93 c	0.89 b
1995[y]	7.9 a	7.86 a	1.01 c

[z] Mean separation within columns by *t test* ($P \leq 0.05$). Data are means of 10 bulbs for SS and 6 bulbs for PA, respectively.

[y] Bulbs harvested in 1995 were analyzed 2-3 months earlier than the previous 4 years.

change of SS:PA ratio is really due to the large change in PA. A change for PA in a year, therefore, would have a very strong effect on onion flavor. Randle (1992) suggested that the strong pungency of an onion can mask the sweet flavor produced by sugars. The results of this multiple year analysis indicates that environmental variables have a strong effect on the final flavor perception of onions.

A Simplified PA Procedure for Determination of PA in a Breeding Program

The SW PA method generally has been used for evaluation of onion pungency by many researchers (Randle, 1992; Lin *et al.*, 1995; and Simmon, 1995). This method, however, is very time-consuming, requiring as long as 100 min for a single test. A simplified procedure was developed which uses much simpler equipment and requires less than 30 min for a test. Two studies were conducted to test the reliability and workability of the MSU PA procedure. A comparison was made in which PA concentrations of onions were tested using the simplified and SW procedures. The PA of five onion cultivars, made up of those considered sweet or pungent, were determined. The second study involved screening two inbred populations 9161 A and 9161 B for high- and low-PA bulbs. Crosses of plants with high PA or of those with low PA were made between 9161 A and 9161 B. If the PA levels of the parent bulbs tested with this procedure are true, the high- and low-PA crosses should then produce the same type of offspring.

Comparison of a Simplified MSU PA Procedure with the Classical Schwimmer and Weston PA Method

PA content measured by the SW method and color intensities measured by the new MSU procedure were highly correlated with a coefficient of determination (r^2) of 0.83. This indicates that 83 percent variation in these tests is accounted for by the linear regression. These results also show that the visual detection with a color value of the final PA-DNPH derivatives (Materials and Methods, p 35) in a sample can be used for estimating PA concentrations. Each color value represents a range of PA concentrations. Figure 11 shows that the color value 1 (yellow) has a PA > 5.4 $\mu mol.g^{-1}$, color value 2 (light purple) covers PA from 5. 4 $\mu mol.g^{-1}$ to 8.22 $\mu mol.g^{-1}$, color value 3 (purple) represents PA from 8.6 $\mu mol.g^{-1}$ to 12.8 $\mu mol.g^{-1}$, and color value 4 (dark purple) covers samples with the highest PA of more than 13.2 $\mu mol.g^{-1}$. For pungency evaluation, the yellow color indicates mildness while the dark color suggests high pungency. This would be more useful for breeders who are more interested in knowing if an onion selection is high or low in PA rather than knowing the exact amount. In addition, it only takes about 25 min to run a group of 25 samples, which makes this method much more time-efficient.

The result of test for background PA, which is unrelated to pungency development in the bulb tissue, shows a very low level for background PA. The same result was previously reported by Yoo *et al.*, (1993). The background

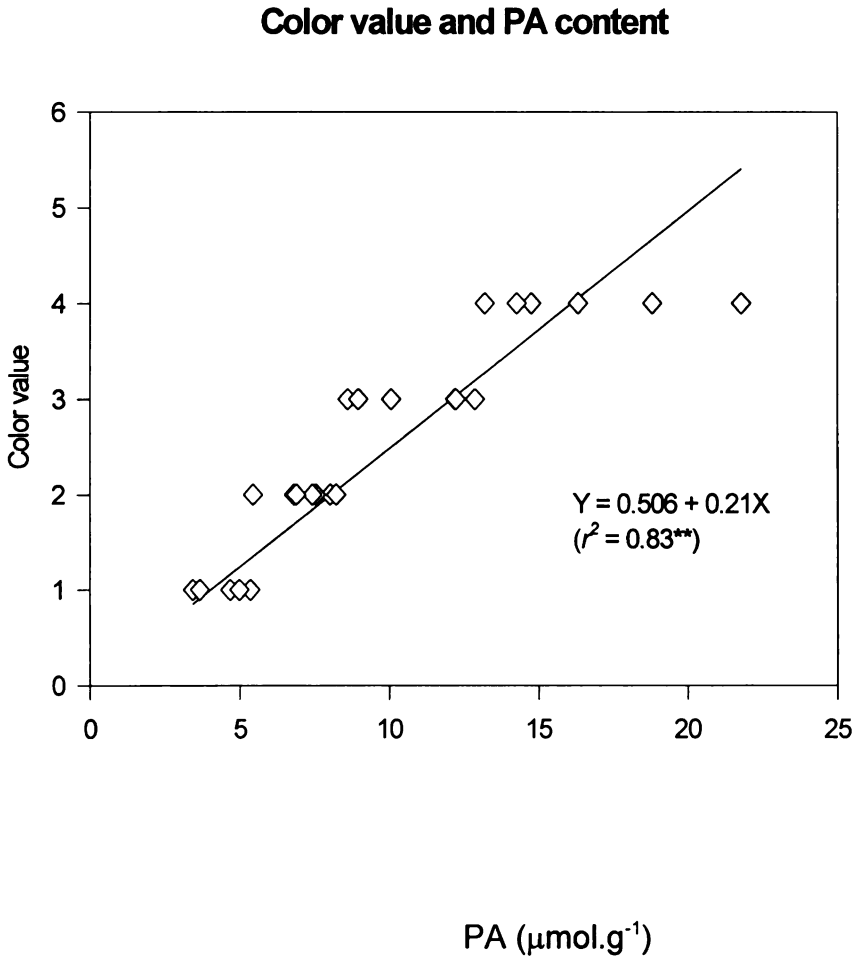

Figure 11. Relationship between the MSU color value procedure and the standard method (Schiwmmer and Weston, 1961). Color value was visually estimated with 4 scales: 1 = yellow, 2 = light purple, 3 = purple, and 4 = dark purple.

PA does not seem to significantly affect the result, and probably can be ignored in breeding programs.

Selection of Two Onion Populations for PA Using the MSU PA Procedure

Progeny from the selections for high- and low-PA parental bulbs from the two inbred populations 9161 A and 9161 B showed a changed PA content compared to the parents (Table 18). High-PA crosses produced progeny, 9506, with a high PA content (14.2 $\mu mol.g^{-1}$) and low-PA crosses produced progeny, 9502, with a low PA content (8.4 $u\mu mol.g^{-1}$). The two new inbred populations differ significantly from each other and the regular selection 9511. These results suggests that the color value procedure could be a reliable tool for a breeding program.

There are some limitations to this procedure. (1) Uniformity of bulb size must be highly considered when comparing different onion varieties since the surface area of the onion slices determine how much PA will develop and be released; (2) A color value does not represent an exact PA level. It only refers to a range of PA in the tissue; (3) Development of PA with DNPH requires a warm temperature. A hot water bath is involved in the SW system to maintain a warm condition for the reaction. Although, this method does not require heating of the samples when the room temperature is around 28 C, low temperature conditions would slow the reaction and would not be suitable for this procedure. It would ,

Table 18. Pyruvic acid content ($\mu mol.g^{-1}$.) of onion bulbs from inbred parents by using the MSU color value procedure.

Lines	Crosses	PA ($\mu mol.g^{-1}$) P1[y]	P2	offspring[x]
9502	9161 A x 9161B	low	low	8.4 a[z]
9506	9161 A x 9161B	high	high	14.2 c
9511	9161 A x 9161B	regular[w]	regular	9.62 b

[z] PA values followed by different letters are statistically different ($P \leq 0.05$). Data are means of 6 bulbs.
[Y] Parental PA, estimated by the new procedure.
[X] Offspring PA, estimated by Schwimmer and Weston method (Schwimmer and Weston, 1961).
[W] Regular selection without regard to PA levels.

therefore, be suggested not to use this method if the temperature during the testing would drop below 25 C.

This method is an alternative to other more complicated systems for onion pungency measurement. Although, it does not result in an exact quantification of PA in onions, it does lead to a quick estimate of onion pungency levels with simple techniques and conventional tools, which often is desired by onion breeders and handlers.

GENERAL DISCUSSIONS

Improvement of onion flavor is a great challenge to onion researchers because there are so many determining factors involved. From the breeding point of view, lower pungency and higher sugar levels in onions may be possible through single-bulb selection and crosses between parent lines high in sugar and low in PA. Environmental factors, however, may not be controlled easily and can offset breeding efforts. Most existing sweet type onions are grown in a particular area where soil, weather, and fertilization factors together provide an optimum environment for the onion to fully develop its unique 'mild taste' potential. Unfortunately, little research work has been directed toward the determination of how to select and breed storable onions with milder flavor.

A few studies including ours have shown that production of onion sugars and PA are genetically determined by dominant-additive genes. To develop milder flavored inbred lines, selection can be conducted effectively within inbred populations for high sugar and low PA bulbs for seed parent use. The means for higher sugar content and lower PA level would be improved, within limits, generation by generation. Significantly increased levels of sugars or soluble solids and reduced PA would be seen after one or two breeding cycles, especially if there is considerable variation within the population.

Considering onion flavor traits of sweetness and pungency to be additive gene-controlled traits, using the crosses of 'high x low' or 'low x high' parent bulb combinations would not be a suggested approach for higher SS and lower PA. The main use of such crosses would be to bring in other desirable characteristics that are lacking in a particular germplasm.

Measurements of onion sugars and pungency must be efficient and reliable. Any successful improvement on the analysis procedures and new tools for bulb screening would be very beneficial to the breeding process. The new method of rapid PA determination developed through this study adds a new option for onion breeders and would make the screening and flavor evaluation processes more efficient.

These studies show that Michigan onions have potential for flavor quality improvement. Compared to the sweet onions, 'Vidalia', 'Texas Super Sweet' and 'Walla Walla', produced in southern and western states, Michigan onions are more pungent, with higher PA levels and are storable. They are actually not low in sugar content. This study suggests that future breeding work to develop sweeter tasting Michigan onions needs more research efforts on redusing PA level rather than on raising the sugar level.

In this study the experimental MSU hybrid 2518-1 and the commercial cultivar Sweet Sandwich produced new encouraging results. New Michigan sweet onions, therefore, may be produced in the future from the parent lines that

have been selected for low PA. More tests are needed to see how stable such hybrids can be in terms of SS and PA levels, and how tolerable they will be to storage effects after bulb pungency is reduced.

The development of high-sugar and low-PA inbred lines might result in higher disease susceptibility and low storability. Low pungency may be accompanied by low tolerance to pathogen attacks (Lin *et al.*, 1995). In this study, some of the newly reselected material from the developed inbreds, including 5718 B and 826 B showed a large number of rotted bulbs during winter storage and suffered considerable field disease attacks. It is not known if these problems are primarily due to inbreeding depression, causing susceptibility, or low pungency and high sugar. Further studies on the relationship between onion flavor and disease tolerance will be necessary to answer these questions. Currently, from the present data, a PA level of 6.0-7.0 $\mu mol.g^{-1}$ possibly would be a level at which severe storage losses could be avoided.

The inheritance of onion pungency and soluble solids can be explained in most cases with a simple additive-dominance model (Lin *et al.*, 1995; Simon, 1995). In the development of a breeding line, selection for a dominant allele in a homozygous condition can be hampered by masking of any recessive alleles. The effect of selection on the genes and genotypic frequency in a population would not be remarkably high over a short breeding time span. The development of gene markers with the use of DNA finger-printing techniques, however, could

improve effectiveness for selection and reduce the time of development of desirable inbreds.

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