Evaluation of safflower seed size, length (shape), and density in relation to seed vigor and oil content

by David Michael Wichman

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
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Abstract:
Stand establishment and weed control are two major problems in dryland safflower production in the Northern Great Plains. Improving stand establishment would improve weed control through competition. Selecting seed that is large and has high density could improve seedling vigor thus improve stand establishment. However, selecting large, high density seed would be selecting lower oil type seed for reproduction.

The purpose of these studies is to evaluate the use of seed size, length (shape), and density of a safflower bulk population in improving seedling vigor while maintaining or improving oil content of the population. To investigate this problem, safflower seed of the 1981 Sidney bulk population, its 1982 progeny grown at Havre, and the cultivars Hartman, S-208, and S-541 grown at several locations, were classified by size, length, density, and evaluated for oil content and seedling vigor. Seed size was based on seed diameter as determined by slotted screens. A laboratory indent cylinder was used to classify seed by length. Seed density classes were obtained using water-alcohol solutions, Caldwell forced air fan, and an Oregon continuous seed blower. Seed oil content was determined by wide-line nuclear magnetic resonance. Emerged seedling weights were used to evaluate seed vigor in field and greenhouse studies.

The large, high density or longer seed, within each seed lot, was found to have more seedling vigor and lower oil content than the small, low density or short seed. Sidney bulk seed in the short class appeared to be more oval in shape and to have less hull in the basal area than the unseparated seed.

The relationship of oil content to seed size, length (shape) and density, when compared across cultivars Hartman, S-208, and S-541, did not follow the same pattern as was found within seed lots. S-541 seed had the highest oil content but it also had the highest seed density and was intermediate to Hartman and S-208 seed in size and length.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for admission to the College of Graduate Studies.

3-3-1983 — Loren E. Wiesner

Date — Chairperson, Graduate Committee

Approved for the Major Department

3/3/83 — Dwane G. Miller

Date — Head, Major Department

Approved for the College of Graduate Studies

3-3-83 — Michael Malone

Date — Graduate Dean

STATEMENT OF PERMISSION TO COPY

Signature David M. Wichman

Date March 3, 1983

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ABSTRACT

Stand establishment and weed control are two major problems in dryland safflower production in the Northern Great Plains. Improving stand establishment would improve weed control through competition. Selecting seed that is large and has high density could improve seedling vigor thus improve stand establishment. However, selecting large, high density seed would be selecting lower oil type seed for reproduction.

The purpose of these studies is to evaluate the use of seed size, length (shape), and density of a safflower bulk population in improving seedling vigor while maintaining or improving oil content of the population. To investigate this problem, safflower seed of the 1981 Sidney bulk population, its 1982 progeny grown at Havre, and the cultivars Hartman, S-208, and S-541 grown at several locations, were classified by size, length, density, and evaluated for oil content and seedling vigor. Seed size was based on seed diameter as determined by slotted screens. A laboratory indent cylinder was used to classify seed by length. Seed density classes were obtained using water-alcohol solutions, Caldwell forced air fan, and an Oregon continuous seed blower. Seed oil content was determined by wide-line nuclear magnetic resonance. Emerged seedling weights were used to evaluate seed vigor in field and greenhouse studies.

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CHAPTER I

INTRODUCTION

Safflower (*Carthamus tinctorius*), an oilseed crop, is well adapted to dryland production in the Northern Great Plains. Its deep-rooting and soil water depletion characteristics make it an ideal crop for use in crop rotations to control saline seep. Research has shown there is little yield benefit when growing safflower on land that was previously fallowed as compared to recropping [9]. Safflower's adaptation to recropping further increases its effectiveness as a tool in saline seep control.

Stand establishment and weed control are two agronomic problems in dryland safflower production in the Northern Great Plains. These problems are interrelated in that the incorporation of pre-plant herbicides loosens the soil which allows the top 5-10 centimeters (cm) to dry. The loose soil and often dry, windy spring conditions at planting time, April 15-May 15, result in a seedbed unsuited for good germination and stand establishment. The weed problem may be enhanced through lack of crop competition.

Safflower cultivars, capable of emerging from deeper planting depth, which are tolerant of nonincorporated herbicides, would improve stand establishment and weed control.

The objective of these studies is to determine if safflower seed (achene) size, specific density and length can be used as selection tools to maintain or improve oil content while selecting for improved seedling emergence.

CHAPTER II

LITERATURE REVIEW

A seed is the product of its genotype and the environment in which it develops. Kidd and West [30] concluded that the external conditions of one generation may influence the performance of the subsequent generation through its effect on the seed.

Halstead [26] postulated that soybean (*Glycine max* L.) seed size is partially determined by the number of seeds in a pod and its position within the pod. Seeds at the top of the soybean pod have higher oil and lower protein content than the other seeds. [16]. Soybean seeds from the bottom of the plant have higher oil and lower protein content than those from the top of the plant [16].

Similar positions effects and seed size relationship occur in safflower. Seed from the primary head (capitulum) are larger and have lower oil content than seeds from secondary and tertiary heads [8,37,52,54]. Mehta et al. [35] concurred with these findings except they reported seed from primary heads had a higher oil content than seed from secondary heads. Reducing the plant population increases the number of secondary and tertiary heads, but lowered the oil content of seed from each position [8,52].

Variations in plant available moisture and soil fertility have a large influence on heads per area and seeds per head while having a minimal influence on seed weight [1,2,6,25,29, 37,41]. Seed size variation within a heterogeneous population is primarily due to genotypes [1,2,5,29]. Mature safflower seed from world-wide origins ranged in weight from 0.03-0.09 grams per seed [51].

Seed Hull

Safflower seed is composed of hull and embryo [46]. The hull component contains 1-2% oil, and is 32-65% of the total weight [15]. Four hull types have been identified based on the development of the inner and outer schlerenchyma cells; normal-hull, partial-hull, thin-hull and striped hull [19,52,45,49]. Sixty percent of the hull in the normal-hull is composed of the highly lignified inner and outer schlerenchymas. The partial-hull, which is recessive to normal-hull, thin-hull, and striped hull are independently inherited hull types in which reduced development of the schlerenchyrmas occur [18,19,33,45,46,49,50].

The expression of some hull types is influenced by the plants' genotype and environment and the seeds' position on the plant. A positive relationship exists between seed size and hull content [8,15,54].

Seed Density

Seed density has long been used as an indicator of seed quality [7]. Test weight has been the primary method of determing seed density.

The oil content of four safflower cultivars was found to be negatively correlated with test weight [38]. A similar association has been observed in the species flax (*Linum asitatissimum* L.), rapeseed (*Brassica* spp.) and soybeans which are capsule, silique, and pod seed types respectively. Seed density has been evaluated as a selection technique for improving oil and protein content in soybeans and safflower [21,22,27,47]. A water-glycerol solution was used to determine soybean seed densities. Selecting plants from low density seed increased the frequency of high oil types while selecting plants from high density seed increased the frequency of high protein types. It was found that seeds with cracked seed coats floated regardless of chemical composition and large seeds had a higher frequency of cracked seed coats [22]. Eslick [21] selected high density safflower seed with forced air and shifted the population toward lower oil and higher protein.

Changes in safflower seed density are associated with other changes in the seed [38]. As seed density increased, air space within the hull decreased, embryo oil content decreased, and hull content usually increased. Seed density increases as hull content increases because the density of the hull is always greater than that of the whole seed. Pawloski [38] also observed that the density of the radicle and the hycotyl was greater than that of the cotyledons. Sudan IV staining showed the cotyledons had larger fat gobules than the radicle.

Several researchers have noted an inverse relationship between oil content and protein content [12,15,17,23,37]. Classen et al. [15] concluded that safflower with 35-40% oil content and 40% or more hull would probably have less than 3.0% nitrogen. The relationship between oil and protein content is not 1:1; therefore, protein can be improved while selecting for higher oil content.

Seedling Vigor

Seed size affects speed of emergence and seedling growth of many species, including monocots, dicots, perennials and annuals. Seedlings from large seeds usually emerge quicker and are more vigorous than those of small seeds [11,13,20,24,31,38,48]. Larger seeds are associated with larger embryo axis, leaf primordia, and cotyledon area [24]. The tendency for large seeds to produce quicker emerging and more vigorous seedlings holds true for the oil seed crops safflower, soybeans and sunflowers [20,28,40,44,48]. Seedlings of large soybean seeds have superior emergence, cotyledon area and more unifoliate leaf area but the seedlings from small seeds have a higher rate of photosynthesis. Further, mixing large and small sized soybeans in a row produced less yield than planting non-mixed lots of large or small seeds [48]. The effect of seed size on seedling vigor may vary by cultivar within a species [3,34].

Researchers have shown that planting large seeds does not always result in quicker or higher percent emergence and can result in decreased speed and percent emergence as compared to an intermediate sized seed [4,10,36,42]. The F4 lines, of a *Vicia sativa* L. X V. *angustifolia* cross, with intermediate seed size were found to be faster emerging and had a higher emergence percentage than the large seeded F4's [4]. The large seeds of alfalfa (*Medicago sativa* L.) and crimson clover (*Trifolium incarnatum*) seed usually produce more vigorous seedlings but sometimes have a lower emergence percentage than intermediate sized seed. It is hypothesized that the cotyledon of some large seeds are over sized [36]. Seed of small and medium sized soybeans cultivars imbibe water and emerge quicker than large seeded cultivars, thus escape soil crusting which can reduce emergence percentage [42]. The pod of sanfoin (*Onobrychis viciafolia* Scop.) delayed water imbition by 4-5 hours, thus delayed germination in laboratory studies. However, no discernible effect was observed in field emergence between shelled and unshelled seed [14]. The benefits of seed vigor may not persist through to yield; however, vigor levels are often reflected in yield of short season annuals [53].

CHAPTER III

MATERIALS AND METHODS

General Procedures

Several seed sources were used in these studies. The 1981 seed production from the world collection of safflower (SB), which has been replanted continuously since 1961 on an irrigated site at the Eastern Montana Research Center, Sidney, Montana, and its 1982 progeny (SB82) grown at the Northern Montana Research Center (NARC), Havre, Montana, are the two bulk populations used in these experiments. Seed of the cultivars Hartman, S-208, and S-541 grown in replicated dryland trials at Huntley, Fort Benton, Havre, and Froid were used in the seed size distribution experiment, the water-alcohol density experiment and the seed size effect on seedling vigor studies. Seed of these three cultivars used in other density experiments and the seed length studies was produced on the Gary Meland farm north of Havre in 1981.

Oil content was determined with a wide-line nuclear magnetic resonance spectrometer [43]. Forty milliliter (ml) seed samples replicated five times in a completely random design were used for oil determination, except for the water-alcohol density separations, where 2 ml samples with six replications were used. Oil content is reported in percent oil content on a dry weight basis.

Seedling vigor was determined by harvesting the above soil portion of the seedling. The seedlings were dried for 24 hour (h) at 60°C then weighed. Weights are reported mg/seedling.

Hull content was determined using 40 seeds with five replications. The seed was dried for 24h at 40°C, weighed, then germinated for 48h at 20°C. The germination percentage was determined, hulls removed, dried for 24h at 40°C and weighed. Hull content is reported in percent hull content of the whole seed on a dry weight basis.

Seed weight was determined by drying four replications of 100 seeds for 24h at 60°C and weighing. Seed weight is reported as mg/40 seed weight to have uniformity with embryo weights.

Field experiments were conducted on a dryland Joplin clay loam site at NARC. The site was fallow for 2 years. The 1982 growing season precipitation was 17.5 centimeters (cm). Nitrogen at 78.5 kilogram (kg)/hectare (ha) and P_2O_5 at 33.5 kg/ha of P_2O_5 were preplant dual injected in the forms of anhydrous ammonium and liquid ammoniated polyphosphate respectively. Trifluralin was preplant incorporated to a depth of 5 cm at a rate of 1.12 kg active ingredient/acre. The seed was treated with the fungicide, zinc ion and manganese ethylene bisdithiocarbamate powder at a rate of 0.8 grams (g)/liter of seed.

The field experiments were planted May 7, 1982, with 19.8 seeds/meter (m) row seeding rate, with 0.3 m row spacing. Seedling vigor evaluations were made 15 and 36 days (d) after planting by harvesting 1.2 m of row from single row plots arranged in a completely random design. The number of replications varied with each experiment. Seed yield samples were harvested 131 d after planting, from 3 row plots, 3.6 m long, in a randomized complete block design with 6 replications.

Greenhouse seedling vigor experiments were grown at 20°C in 25 × 50 × 7.5 cm flats with one replication/flat. Seed was planted 2.0 cm deep and all seedlings were harvested 21 days after planting. A randomized complete block design was used with 12 seeds/replication and 5 replications/experiment.

Seed Size

Seed size distribution of Hartman, S-208, S-541 and SB was determined by sieving 0.9 liters of seed through a series of slotted screens in the following order 4.0, 3.6, 3.2, 2.8, and 2.4 mm size. Seeds which did not pass through a particular screen were classified as that screen size. Seed smaller than 2.4 mm was discarded. The seed size distribution of the cultivars Hartman, S-208, and S-541 was replicated five times with seed source locations serving as replications. Seed size distribution of the SB82 seed lots was divided into three classes; greater than 3.2, 2.8, and less than 2.8 mm. Seed size distribution and oil content of progeny of SB 2.8, 3.2 mm, and unsized parents was determined on seed produced in single row plots, which were planted 2.5 cm deep, in a completely random design with six replications. The effect of SB seed size class on seedling vigor was evaluated in two separate experiments, one planted 2.5 cm deep and the other 6.25 cm deep. The seed was planted in single row plots in a completely random design with six replications. Field experiments to evaluate seed size effect on seedling vigor of Hartman, S-208, and S-541 were planted 6.25 cm deep in single row plots with 10 replications (5 seed source locations).

Seed Density

Seed density classifications in these experiments is based on relative seed density. Three seed density classification methods were evaluated; water-isopropyl alcohol solution (W-A), forced air from a 23 cm Caldwell fan CFA, and forced air in an Oregon continuous seed blower (OCSB).

Water-alcohol solutions with alcohol concentrations ranging from 65, 45, 25 and 0 percent by volume were used to divide SB 2.8 (7/64) and 3.2 mm (8/64) sized seed into five density classes. The density separations were designated as light, mid light, mid, mid heavy, and heavy. The seed was first exposed to the high alcohol concentrations. Seed which sank was then exposed to solution lower alcohol concentrations. Therefore, high density seed was exposed to all four W-A density solutions. SB 2.4 (6/64), 3.6 (9/64), and 4.0 mm (10/64) sized seed was split into high and low density classes with 45 percent W-A. The alcohol concentration for density classifications of Hartman, S-208, and S-541 was that which gave a 50-50 split into high and low density by volume of the seed produced at Fort Benton (Table 1). The seed density classes obtained were used to determine the effect of seed density on oil content and seedling vigor. Seedling vigor was evaluated by field planting seed density classes 6.25 cm deep in single row plots in a completely random design. Seed density studies using the SB seed sources had 6 replications (2 sizes) and the studies using cultivars had 10 replications (5 seed source locations).

Table 1. Isopropyl Alcohol Percentage in Water-Alcohol Solution Used to Determine Seed Density Classes of Three Safflower Cultivars.

Cultivar	Alcohol Concentration %
Hartman	62
S-208	54
S-541	45

Forced air from a 23 cm Caldwell fan (CFA) was used to separate SB unsized, SB long, and SB mid length seed into four density classes. The seed was poured through the CFA air stream and caught in 4 (60 cm × 60 cm × 30 cm) boxes placed under the flow of air. The first box was placed against the base of the fan to insure all seed was caught. Seed falling in the box closest to the fan was classified as high density, followed mid high, mid low, and low density seed. CFA separated seed was used to evaluate the relationship of seed density and oil content.

The Oregon continuous seed blower model CS-1 (OCSB) was used to separate SB, unsized, SB 3.2 mm, SB82, Hartman, and S-208 seed into five density classes: low, mid low, mid, mid high and high by opening the intake aperture; 7.5 8.0, 8.5, and 9.0 cm respectively. The effect of seed density, as determined by OCSB, on seedling vigor was evaluated in greenhouse studies.

Seed Length

The Seed Master laboratory cleaner-indent cylinder was used to make length separations on seed of SB, SB82, Hartman, S-208 and S-541. Two kg of seed were first processed using the No. 20½ indent cylinder (indents 8 mm in diam). Seed that was not picked up was classified as "long." The seed picked up by the No. 20½ indent cylinder was reprocessed using the No. 11 indent cylinder (indents 5 mm diam). The seed which was picked up by this indent cylinder was classified as "short" and seed not picked up was classified "mid." SB82 had an additional length class, " mid short," which was seed that remained in the indent cylinder. Two hundred seeds of each length separation were measured using a binocular microscope at 15X to determine seed length in millimeters. Field experiments to evaluate the effect of seed length on seedling vigor were planted 2.5 cm deep in single row plots with six replications. Seedling vigor was determined by harvesting seedlings from 1.2 m of row, 36d after planting.

The effect of seed length on seed yield was evaluated by planting seed 2.5 cm deep in three row plots arranged in a randomized complete block design and harvesting 3.6 m of all three rows.

CHAPTER IV

RESULTS AND DISCUSSION

Seed Size Distribution

Most seed of the S-208 and S-541 cultivars was of the 2.8 and 3.2 mm size as compared to Hartman which had smaller seed and the majority of it was in the 2.4 to 2.8 mm size classes (Table 2). The 1981 Sidney bulk population (SB) seed size distribution is similar to S-208 and S-541 cultivars, with 84.1% of its seed in the 2.8 to 3.2 mm size (Table 3).

Table 2. Average Seed Size Distribution of Three Safflower Cultivars Grown at Five Locations.

	Screen Size (mm)			
Cultivar	2.4 %	2.8 %	3.2 %	3.6 %
Hartman	25.9 a†	64.2 a	9.6 a	0.3 a
S-208	3.1 b	43.4 b	49.9 b	3.5 b
S-541	7.1 b	54.3 a	37.3 c	1.3 a

†Means in the same column followed by a common letter are not significantly different based on Newman-Keul test of significance at the .05 probability level (P = .05 N.K. test).

Table 3. 1981 Sidney Bulk Safflower Population Seed Size Distribution and Oil Content of Each Size.

	Screen Size (mm)				
Seed Character	2.4	2.8	3.2	3.6	4.0
Percentage of pop. (%)	3.7	35.3	48.8	10.9	1.3
Oil content (%)	31.2 a†	28.9 b	27.8 b	25.7 c	24.4 c

†Means in same row followed by a common letter are not significantly different (P = .05 N.K. test).

Seed size distribution of the SB progeny is influenced by the seed size of the parents. Progeny of the SB 3.2 mm seed had 8.6% more seed larger than 2.8 mm size and 5.6% less seed of the 2.8 mm size than did the progeny of the 2.8 parent (Table 4).

Table 4. Average Seed Size Distribution of Progeny of 1981 Sidney Bulk Safflower (SB) Seed Length and Size Classes Grown at Havre in 1982.

Parent Screen Size	Screen Size (mm) < 2.8 %	2.8 %	⩾ 3.2 %
SB 2.8 mm	11.5 a†	64.5 a	23.8 a
SB 3.2 mm	8.3 b	58.9 b	32.4 b
SB unseparated‡	12.5	63.7	26.7

†Means in the same column followed by a common letter are not significantly different (P = .05 N.K. test).
‡Not included in statistical comparison.

Further, the seed distribution of the progeny of SB unsized parent is similar to that of progeny of the SB 2.8 mm seed (Tables 4 and 31). The effect of environment on seed size distribution is demonstrated by comparing the seed size distribution of SB, which was grown under irrigation to that of SB82, which was grown under dryland conditions. The majority of the SB seed was of the 3.2 mm or larger size while the majority of the SB82 seed is of the 2.8 mm size (Table 5).

Table 5. Seed Size Distribution of 1981 Sidney Bulk Population (SB) and Its 1982 Progeny (SB82).

Seed Source	Screen Size (mm) < 2.8 %	2.8 %	⩾ 3.2 %
SB	3.7	35.3	61.0
SB82	12.5	63.7	26.7

*No statistical comparison.

Oil Content

The trend for lower oil content with increasing seed size occurred in all safflower evaluated. There was a 6.8% decrease in oil content from the 2.4 mm seed size to the 4.0 mm seed, of Hartman, S-208, and S-541, which was grown at five locations, had a higher oil content than the 3.2 mm seed 13 out of 15 times (Tables 28, 29, and 30).

Seed sizing as a selection technique to improve oil content has limitations. These limitations become obvious when seed size and oil content of Hartman, S-208, and S-541 are compared. Hartman has the smallest seed yet has the lowest oil content (Tables 2 and 6). Whereas, S-541 has the highest oil content and is intermediate in size. Further, the oil contents of progeny of SB 2.8, SB 3.2 mm and SB unsized were 28.2, 28.2 and 28.4 percent respectively, which was not significantly different (P = .05 N.K. test).

Table 6. Average Oil Content of Three Cultivars Grown at Five Locations in 1981.

Cultivar	Hartman	S-208	S-541
Oil content†	41.5	43.7	45.7

†Mean oil content value obtained from size and density separations, Tables 28, 29, and 30.

Seedling Vigor

Larger safflower seeds produced more vigorous seedlings which concurs with previous research [20]. In field studies at Havre, SB 3.2 mm seed planted 6.25 cm deep, produced heavier seedlings than SB 2.8 mm seed 36 days after planting (Table 7). Similar results were obtained at the 2.5 cm planting depth (Table 8). SB 3.2 and 3.6 mm seed, planted 2.5 cm deep produced significantly heavier seedlings 36 days after planting than the SB 2.8 mm seed. However, there was no difference in the number of seedlings per 1.2 meters of row. The reduced seedling vigor due to small seed size did not result in reduced yield (Table 9). These results are similar to those obtained with sunflowers [40].

Table 7. Field Performance of Seedlings of 1981 Sidney Bulk Safflower Seed Size Classes Planted 6.25 cm Deep at Havre in 1982.

	Days After Planting			
	15		36	
Seed Size	plts/1.2 m	mg/plt	plts/1.2 m	mg/plt
SB 2.8 mm	22.3 a†	18. a	23.7 a	171 a
SB 3.2 mm	21.7 a	17. a	23.2 a	240 b

†Means in the same column followed by a common letter are not significantly different (P = .05 N.K. test).

Table 8. Field Performance of Seedlings of 1981 Sidney Bulk Safflower Seed Size Classes Planted 2.5 cm Deep at Havre in 1982.

	Days After Planting	
	36	
Seed Size	plts/1.2 m	mg/plt
SB 2.8 mm	19.5 a†	290 a
SB 3.2 mm	19.3 a	347 bc
SB 3.6 mm	22.2 a	404 c
SB unseparated	19.5 a	339 ab

†Means in the same column followed by a common letter are not significantly different (P = .05 N.K. test).

Table 9. Seed Yield of 1981 Sidney Bulk Safflower Population Planted 2.5 cm Deep and Thinned to 39 Plants per Square Meter at Havre in 1982.

Seed Size	kg/ha
SB 2.8 mm	1517
SB 3.2 mm	1538
SB 3.6 mm	1544
SB unseparated	1550

No significant differences (P = .05 N.K. test).

Larger seed of Hartman, S-208, and S-541 also produced heavier seedlings, though the difference due to seed size was not significant (Table 10). Low plant number, which may be due to reduced viability from exposure to the water-alcohol solution could have caused enough variability to render the difference nonsignificant.

Table 10. Average Seedling Weight Produced at Havre in 1982 by Sized Seed of Three Cultivars Grown at Five Locations.

	Days After Planting			
	15		36	
Cultivar	2.8 mm mg	3.2 mm mg	2.8 mm mg	3.2 mm mg
Hartman	.013 a†	.014 a	.056 a	.062 a
S-208	.016 a	.015 a	.076 a	.083 a
S-541	.013 a	.018 a	.048 a	.067 b

†Means within the same row and harvest date followed by a common letter are not significantly different (P = .05 N.K. test).

These data show that selection of large seed for planting will improve seedling vigor thus should be beneficial in stand establishment. The selection of larger seeds for planting should improve the oil content of the seed lot from which the large seed were removed, thus improving the quality of seed to be processed. Seed sizing should not be done for too many generations, especially on cultivars which are composites of several lines or early generation releases, because the genetic character of the cultivar could be altered.

In a breeding program, the selection of large seeded safflower to improve seedling vigor should have to be combined with other selection criteria to prevent simultaneous selection toward lower oil content.

Seed Density

Evaluation of Methods

The three methods of making density separations, water-alcohol solution (W-A), Caldwell forced air fan (CFA), and Oregon continuous seed blower (OCSB), are all effective in grouping safflower seed into density classes (Table 11). However, the three methods are not equal in their ease of use or precision.