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RESEARCH ARTICLE

Influence of harvest date on seed yield and quality in forage kochia

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Abstract Forage kochia (*Bassia prostrata*) is used for rangeland reclamation and livestock and wildlife forage, but limited research has been conducted on its seed production. Therefore, this research evaluated the effect of harvest date on seed weight, germination, and seed yield of forage kochia subspecies *virescens* and *grisea*. Seed was harvested from individual plants for 3 years during October, November, and December. October harvest had the lightest 100-seed weights, with the November harvest slightly heavier than December, for most accessions. Cultivar Snowstorm and breeding line Sahsel, both subsp. *grisea*, had the greatest 100-seed weights in November, 155 and 143 mg, respectively, whereas, cv. Immigrant (subsp. *virescens*), the standard for forage kochia, ranked among the least for 100-seed weight. For most accessions, germination was lowest from the October harvest (11%–43%), with greater germination with November and December harvested seeds (43%–64%). Viable seed yields were greatest in November with the exception of two accessions, which peaked in October, indicating earlier maturity. Results indicate that forage kochia usually reaches optimum seed maturity by early November, after plants are exposed to freezing temperatures; however, earlier maturing accessions exist in both subspecies *virescens* and *grisea*.

Keywords forage, rangeland, seed germination, seed quality

1 Introduction

Western rangelands in the USA have increasingly become degraded due mainly to climate fluctuations, wildfires, overgrazing and the invasion of non-native annual plant species. The success or failure of subsequent restoration and revegetation efforts of forbs and shrubs on these rangelands depends partially on acquiring sufficient quantities of quality seed^[1]. Often seeds of forbs and shrubs do not ripen evenly and show differences in maturity among subspecies, among plants within a subspecies, and even among different stems within individual plants^[2]. Therefore, when selecting a harvest date, seed producers must compromise between seed maturity and loss of yield due to shattering^[2].

Forage kochia (*Bassia prostrata*; previously *Kochia prostrata*), is a semi-evergreen, subshrub in the Chenopodiaceae comprised of the two subspecies; subsp. *virescens*, a green plant type, and subsp. *grisea*, a gray plant type^[3]. Forage kochia is an important forage in its native environment of Eurasia, where it is utilized by sheep, goats, camels and horses^[4]. Waldron et al.^[5] recommended the use of forage kochia in western USA, as it is well adapted to these semiarid to arid rangelands, and increases nutritional value, carrying capacity and livestock performance, especially for autumn/winter grazing. Furthermore, forage kochia is reported to have high salt and drought tolerance^[4,6,7], and has been shown to have potential to rehabilitate disturbed rangeland areas where frequent wildfires occur and invasive annuals, such as *Halogeton glomeratus*, displace native perennials^[8–11]. Forage kochia was first introduced into the USA in the early 1960s and subsp. *virescens* cv. Immigrant, was subsequently released in 1984 after evaluations identified it as a palatable forage that had exceptional ability to establish, persist and reduce soil erosion on disturbed rangelands in the Intermountain

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West^[12,13]. Immigrant is now estimated to be established on over 202000 hm² in the western USA^[14]. More recently (2012), cv. Snowstorm was the first cultivar of subsp. *grisea* released in the USA and, when compared to Immigrant, is a taller and larger-statured cultivar with enhanced winter grazing attributes^[15].

Forage kochia can be difficult to establish reliably, in part because of its seed biology^[16]. Germination controls in forage kochia include both seed dormancy^[3] and asynchronous germination^[17], which are believed to be traits that enhance establishment in harsh environments by ensuring differential germination under favorable conditions^[16]. Importantly, Waller et al.^[18] suggested that these germination controls likely develop near the end of forage kochia seed ripening. The period of seed ripening of forage kochia is highly variable^[3] and is affected by the weather, with freezing temperatures hastening seed maturation^[12,19]. Moreover, depending on the subspecies, seed ripening begins as early as late September and can continue to early December^[20,21]. Seed also ripens unevenly (up to 30 d) across individual plants of the same subspecies, and can even vary on the same raceme^[3]. Given the variability of ripening between plants and subspecies, combined with the effect of ripening on germination controls, understanding the appropriate harvest date that maximizes seed viability and yield is critical. Therefore, a study was designed to document maturity differences among accessions and subspecies of forage kochia, and to elucidate the effect of harvest date on seed yield and seed quality. This information will improve the quality of harvested forage

kochia seed, and thereby increase the likelihood of successful establishment of rangeland plantings.

2 Materials and methods

The forage kochia accessions used herein were established in a research nursery plot in 2006 at the Utah Agriculture Experiment Station Blue Creek Research Farm (41°46'0.6" N, 112°26'16.8" W, 1582 m). The site is located about 24 km north-west of Tremonton, UT, USA with soil classified as Parley's silt loam (fine-silty, mixed, mesic, Calcic Argixerolls). During the three years seed was harvested for this study (2009–2011), the average annual precipitation was 30 cm with 40% of the total precipitation falling from April to August when forage kochia is actively growing. Seven accessions of forage kochia, representing three ploidy levels (2×, 4×, and 6×) in both subspecies (*virescens* and *grisea*), and the commercially available, cvs Immigrant and Snowstorm, were examined (Table 1). Forage kochia seedlings were transplanted from cone-tainers (Ray Leach Cone-tainer Cells, Stuewe and Sons Inc., Tangent, OR, USA) into 10-plant plots in a randomized complete block design with four replicates. The plant spacing was 1 m between rows and 0.5 m between plants within rows. Thus, there were a total of 40 plants of each accession, and the close proximity of plants allowed sufficient cross-pollination to evaluate seed production traits. The nursery was surrounded by a border row of Immigrant to eliminate edge effects.

Table 1 Forage kochia accessions used to evaluate the effect of harvest date on seed yield and quality in Box Elder County, UT, USA

Subspecies	Accession	Ploidy ^a	Status	Origin	Reference
<i>grisea</i>	Snowstorm	4×	Cultivar	Uzbekistan	[15]
<i>grisea</i>	Sahsel	4×	Breeding population	Uzbekistan	[22]
<i>grisea</i>	KZ6Xsel	6×	Breeding population	Kazakhstan	[23]
<i>grisea</i>	U-20	6×	Collection	Russia	[24]
<i>virescens</i>	Immigrant	2×	Cultivar	Russia	[13]
<i>virescens</i>	Pustsel	2×	Breeding population	Uzbekistan	[22]
<i>virescens</i>	BC-118	2×	Breeding population	Uzbekistan	unpublished

Note: ^a Base chromosome number of $n = 9$.

Table 2 Growing degree days (GDD) corresponding to seed harvest dates of forage kochia in Box Elder County, UT, USA

Month	2009		2010		2011	
	Date	GDD ^a	Date	GDD	Date	GDD
October	7	1957	6	1904	5	1925
November	4	2016	3	2007	3	1994
December	2	2030	8	2022	7	1994

Note: ^a Cumulative growing degree days using a base minimum temperature of 5.56°C.

Seed harvests were conducted in the first week of October, November, and December in 2009, 2010, and 2011, respectively. The cumulative growing degree days (GDD) for each harvest, using a base minimum temperature of 5.56°C, are given in Table 2. At each harvest, three plants from each plot were harvested individually by cutting and bagging all seed bearing stems. The same three plants were used each year for each respective monthly harvest. The bags were placed inside a greenhouse to air dry until the seed could be easily stripped from the stems. The stripped seed and remaining small stems, leaves and chaff were further air-dried to 3% moisture content before threshing (Wintersteiger LD 180 st4 sample thresher, Wintersteiger, Inc., Salt Lake City, UT, USA) to separate seed still remaining on small stems and to remove utricles from seed. The seed was sifted to remove debris larger than the seed, and then samples were cleaned using a seed blower (Carter Day International, Style# CFZ1, Carter Day International, Inc., Minneapolis, MN, USA) to remove additional chaff. If additional cleaning was needed to remove inert material, seed was blown manually using a column seed cleaner (Agriculex CB-1 Column Seed Cleaner, Agriculex Inc., Guelph, ON, Canada). Following seed cleaning, seed from each harvest was weighed to determine total yield for each plant harvested. Subsamples of seed from each plant were manually separated from any remaining foreign material, counted by hand into three replicates of 50-seeds and weighed.

After seed preparation, germination boxes (Acrylic Germination Boxes, 11 cm, Cont 156C, Hoffman Manufacturing Co., Jefferson, OR, USA) with two layers of blotter paper (Number 3, Steel Blue Germination Seed Blotters, 10 cm × 10 cm) positioned in the bottom of the boxes were saturated with deionized water, and the 50-seed subsamples of each treatment were arranged on top of the blotter paper. Seeds were spaced so that they were not in contact with one another. Each box was treated with two sprays from a spray bottle of diluted commercial bleach (0.05% sodium hypochlorite final concentration) that served as a fungicide. The boxes were covered and arranged in a randomized complete block design in an incubator to pre-chill seed samples at 2.5°C for 14 d, which allowed the seeds to imbibe moisture, and overcome dormancy and asynchronous germination. After pre-chilling, the temperature was increased to 20°C for 7 d. This germination procedure adheres to the standards set by the Association of Official Seed Analysts for forage kochia^[25]. Following incubation, counts were made to determine the number of germinated seeds, in which seeds were considered germinated when the radical had extended 5 mm. Total viable seed produced per plant was then calculated by multiplying total seed produced by the germination percentage.

The average values for the three plants within each accession-replicate combination were used for statistical

analyses. All data were analyzed using the Proc Mixed procedure of SAS^[26] with replication as a random variable, and accession, month and year as fixed effects. Year was considered a repeated measure and the appropriate covariance model (CS) was used^[26]. Mean comparisons were made among treatments using Fisher Protected LSD tests at $P = 0.05$ ^[27]. Most interactions with year were not significant, and the few that were significant were due to magnitude. However, accession × month of harvest interactions were statistically and biologically significant for all traits, therefore LSDs for all traits were calculated from the error term associated with the accession × month means. Pearson's correlations among seed traits were calculated using the CORR procedure of SAS.

3 Results

3.1 100-seed weight and germination

In this study, there were low, but significant, correlations between germination and seed yield ($r = 0.21$, $P = 0.007$), and 100-seed weight and seed yield ($r = 0.21$, $P = 0.003$). On average, October-harvested seed ranked lowest in 100-seed weight and November-harvested seed was significantly ($P \leq 0.05$) or trended ($P \leq 0.10$) slightly higher than December for all accessions, except population KZ6Xsel (Fig. 1). Immigrant, long considered the standard of forage kochia, ranked among the lowest in 100-seed weight (45.5, 93.9, and 87.0 mg in October, November, and December, respectively) (Fig. 1). Snowstorm and the experimental population Sahsel, both subsp. *grisea*, had greater 100-seed weights than other accessions with peak weights in November, 154.7 and 143.3 mg, respectively (Fig. 1). Both are tall-type, large statured ecotypes, when compared to Immigrant, which is relatively prostrate. The *grisea* accession with the least seed weight in November was population KZ6Xsel (79.7 mg), which also ranked least in this trait among all accessions in November (Fig. 1). In contrast, population BC-118 had the greatest November 100-seed weight of the three *virescens* accessions, with a mean of 123 mg compared to population Pustsel (107 mg) and Immigrant (93.9 mg) (Fig. 1).

Germination was measured as a means to determine the viability of the seed harvested. Similar to the 100-seed weights, germination in October ranked lowest when compared to other months for all accessions, except population KZ6Xsel (Fig. 1). The only statistical difference between November and December harvest dates was detected in population BC-118, where germination in December (64.2%) was greater than November (48.4%) (Fig. 1). As was observed for seed weight, germination of population KZ6Xsel was unique in that there were no significant germination differences detected between seed

harvested in October (60.4%), November (51.2%), and December (60.3%) (Fig. 1).

3.2 Total and viable seed yield

Mean seed yield per plant was variable among accessions and harvest dates (Fig. 2). In general, peak total seed production was reached for most accessions in November, with the exceptions of accessions KZ6Xsel and Pustsel, which had peak seed production in October (Fig. 2), indicating that they mature earlier. Harvesting Immigrant during November resulted in the greatest total seed yield (20.3 g), though not significantly higher ($P > 0.05$) than the November harvest for population BC-118 (17.0 g), Snowstorm (14.8 g), and the October harvest of Pustsel (15.1 g) (Fig. 2).

The quantity (g per plant) of viable seed (total seed ×

percent germination) for each harvest and accession was calculated to better understand the association between yield and maturity of forage kochia seed harvested on different dates. Figure 2 illustrates the trend over time for total seed yield and corresponding viable seed yield by accession. Immigrant had the greatest ($P \leq 0.05$) viable seed yield among the accessions in November (11.8 g) and was among the highest in December (7.2 g), though not significantly different to nine other accession/month combinations including December harvests of populations U-20 (3.7 g) and Sahsel (3.6 g) (Fig. 2). Populations KZ6Xsel and Pustsel were the only two accessions with greater viable seed yield in October (8.7 and 4.7 g, respectively) than the following months of November (2.1 and 3.9 g, respectively) and December (1.6 and 1.2 g, respectively) (Fig. 2). The remaining accessions manifested similar trends to those detected for total seed

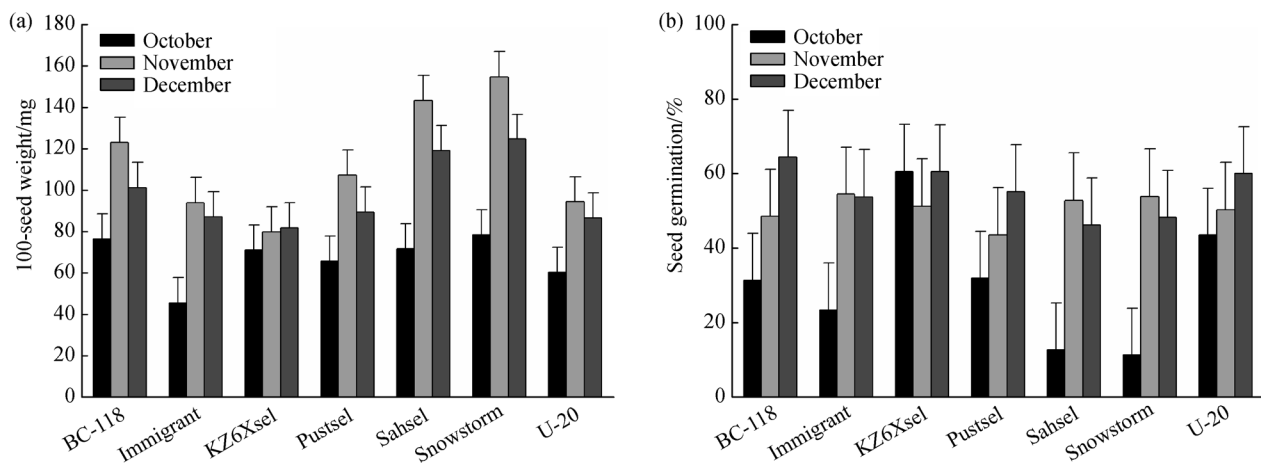


Fig. 1 Mean response of 100-seed weight (mg) and germination (%) of seven accessions of forage kochia harvested in October, November, and December in 2009, 2010, and 2011, respectively. Bars represent the LSD ($P = 0.05$) value of 13.4 and 15.1 for 100-seed weight (a) and germination (b), respectively, based upon the error term associated with the accession × month means.

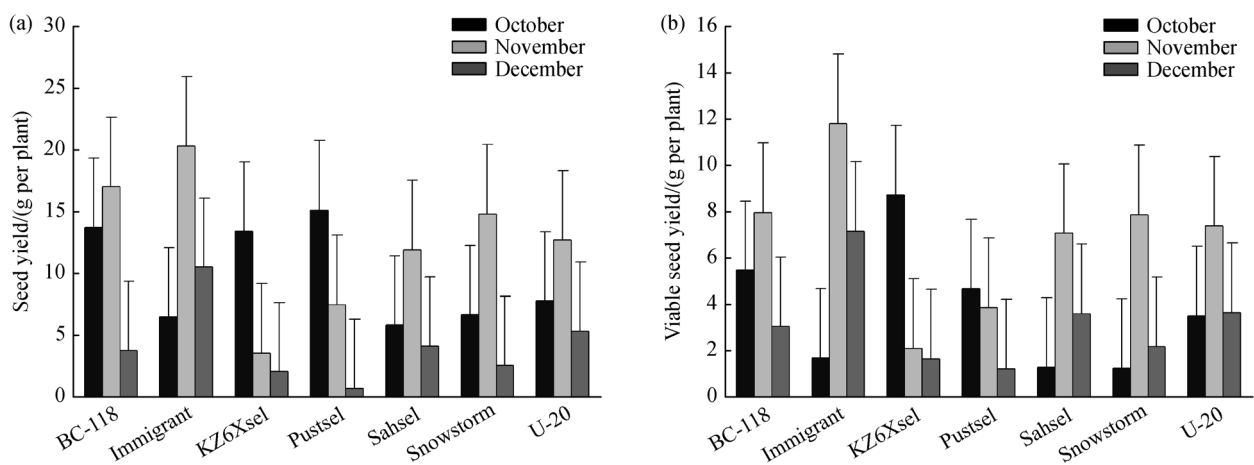


Fig. 2 Mean response of total seed yield (a) and viable seed yield (b) (g per plant) of seven accessions of forage kochia harvested in October, November, and December in 2009, 2010, and 2011, respectively. Bars represent the LSD ($P = 0.05$) value of 5.6 and 3.3 for seed yield and viable seed yield, respectively, based upon the error term associated with the accession × month means.

production, peaking in November, and declining in December. These data indicate that by December, seed had fully matured and had often shattered resulting in reduced total and viable seed yield (Fig. 2).

4 Discussion

Factors influencing the germination of forage kochia are of interest because of the difficulty of maintaining seed viability and subsequently establishing forage kochia on rangelands^[17,28]. Although several studies have investigated the effect of harvest date on seed germination^[18,29], they included limited within- and between-subspecies comparisons. Stewart et al.^[29] compared seed harvest dates with Immigrant on two-week intervals from early-October to mid-November. Data presented herein corroborate their findings, which showed that early- and mid-November harvests resulted in comparatively greater germination in Immigrant. They, however, did not evaluate subsp. *grisea* or other accessions within subsp. *virescens*. Waller et al.^[18] compared seed germination of one accession of *virescens* with one accession of *grisea* when harvested at 10-d intervals from early-October to mid-November. They reported that *grisea* had greater germination than *virescens* at almost all harvest dates, with *grisea* germination peaking on October 30, as opposed to October 20 for *virescens*. In contrast, no distinct differences for peak germination between the two subspecies were detected in the present study, where *virescens* germination peaked for seed harvested between the first weeks of November and December. Seed weight and germination were not statistically correlated ($r = 0.12$, $P = 0.109$). This is in contrast to findings by Bai et al.^[30], who found that heavy Wyoming big sagebrush (*Artemisia tridentata* subsp. *wyomingensis*) seeds germinated more rapidly and at higher rates than lighter seeds. Nevertheless, results herein corroborates the reported lack of correlation between seed weight and germination in western wheatgrass (*Pascopyrum smithii*)^[31], and lack of correlation between seed weight and seedling establishment in forage kochia^[15]. Further studies are needed to elucidate the relationship of seed weight, germination and seedling establishment within forage kochia accessions as opposed to comparisons among accessions and subspecies.

It was not surprising that Immigrant was the greatest seed yielder as it was originally selected for release partly due to its high seed production^[13]. The December harvest produced the least seed yield for all accessions, probably due to mature seeds shattering and falling to the ground prior to harvest (Fig. 2). However, Immigrant has been observed to be less prone to shattering^[21], which was also supported by it having the greatest December seed yield (Fig. 2). Seed producers and the USDA-NRCS Plant Guide^[19] suggest a hard frost is required for good seed set in forage kochia. During the course of this study, the

first hard frosts occurred October 1, 2009, and October 25, 2010 and 2011. The first hard frost in 2010 and 2011 was only 7 to 8 d prior to the November seed harvests, and thus, supports this recommendation. However, the variability observed among accessions also suggests genetics have a large role in determining when seed set occurs.

Seed yield and viability parameters in any seed crop are associated with the stage of maturity at which the seed is harvested^[32]. For indeterminate species, the ideal harvest time is immediately before the loss of mature seeds exceeds the amount of seeds yet to reach maturity^[33,34]. Balyan^[3] reported uneven ripening among plants within forage kochia accessions, and in this study it was observed that forage kochia accessions demonstrated high variability not only among accessions, but among individual plants (Fig. 3). Although Waldron et al.^[21] proposed that due to its indeterminate nature, forage kochia ripening might not be complete until early December; the results presented herein suggest sufficient ripening can occur prior to December. The uniformly low December viable seed yields (Fig. 1) is in stark contrast to the high percent germination of December seed (Fig. 2). Therefore, contrary to the results of Stewart et al.^[29] and Waller et al.^[18], these results suggest that germination per se is inadequate to determine the maturity or the best seed harvest date for indeterminate species like forage kochia. The variation within accessions for seed germination and viable seed yield (Fig. 3; Fig. 4) also indicate that it should be possible to develop early and late maturity breeding populations. Selecting and breeding forage kochia populations with more uniform seed maturity could ease the difficulty of selecting a harvest date, and would allow for higher yield and viability than is currently possible.

5 Conclusions

This research compared the affect accession and harvest date had on germination, 100-seed weight, and total and viable seed yield of forage kochia. Harvesting forage kochia during the early maturing phase resulted in poor yield and viability due to the high number of immature and undeveloped seeds. However, delaying harvest until December when all seeds had reached maturity greatly reduced viable seed yield due to shattering. Given the high variability observed for seed yield, 100-seed weight and germination, our results suggest that selection for reduced variability within populations would simplify the task of choosing an appropriate harvest date and increase viable seed yield. In conclusion, optimum month of seed harvest was variable among experimental populations of forage kochia, and there were no trends that uniquely distinguished subspecies *grisea* and *virescens*. However, the data indicate that both Immigrant and Snowstorm should be harvested in mid-November to maximize viable seed production.

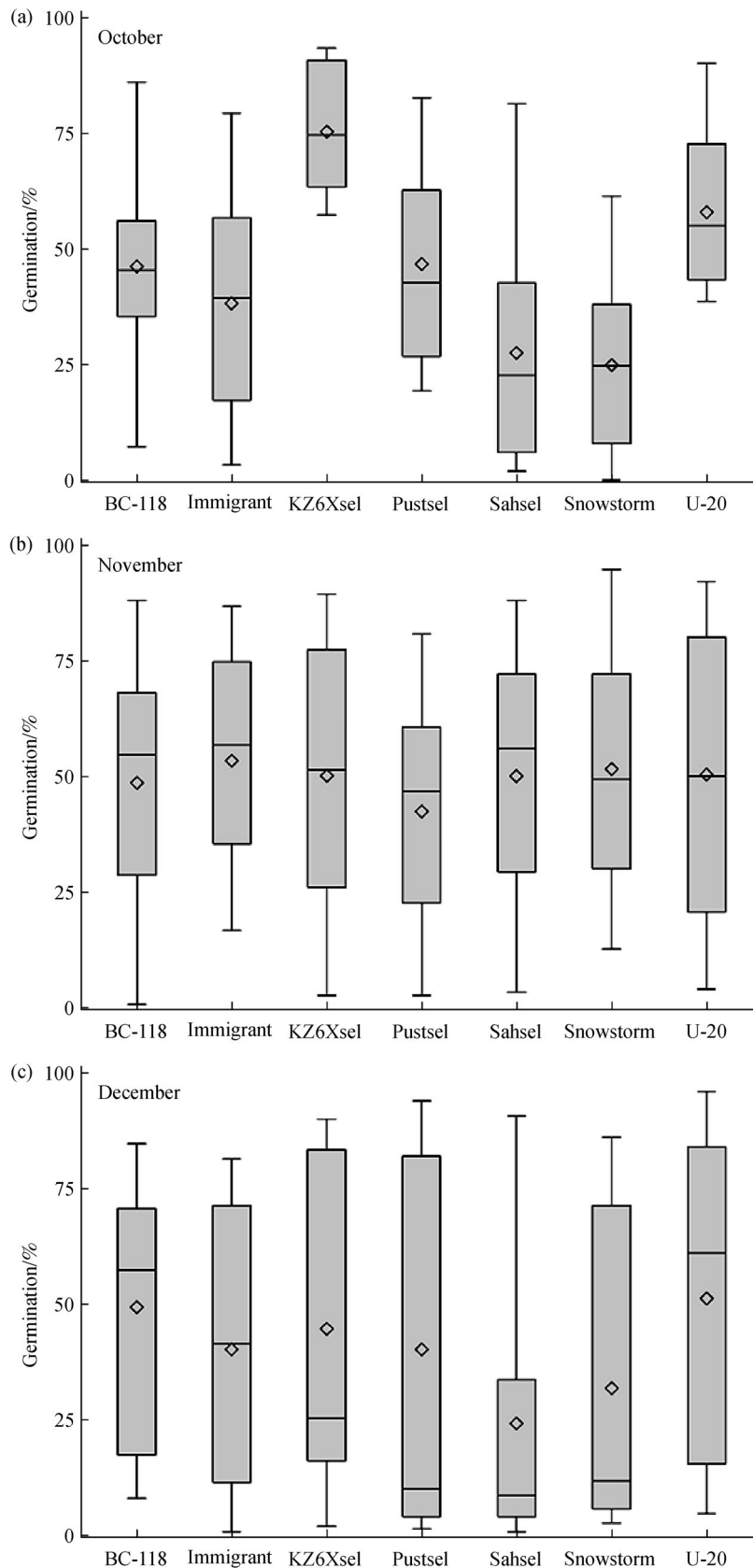


Fig. 3 Skeletal boxplot graphs of the variability in seed germination (%) among individual plants of seven forage kochia accessions harvested in the first week of October (a), November (b), and December (c) in 2009, 2010, and 2011, respectively. Whiskers represent the extreme range of values. The top and bottom of each box is the 75th and 25th percentile, respectively, and diamonds and lines represent the mean and median, respectively.

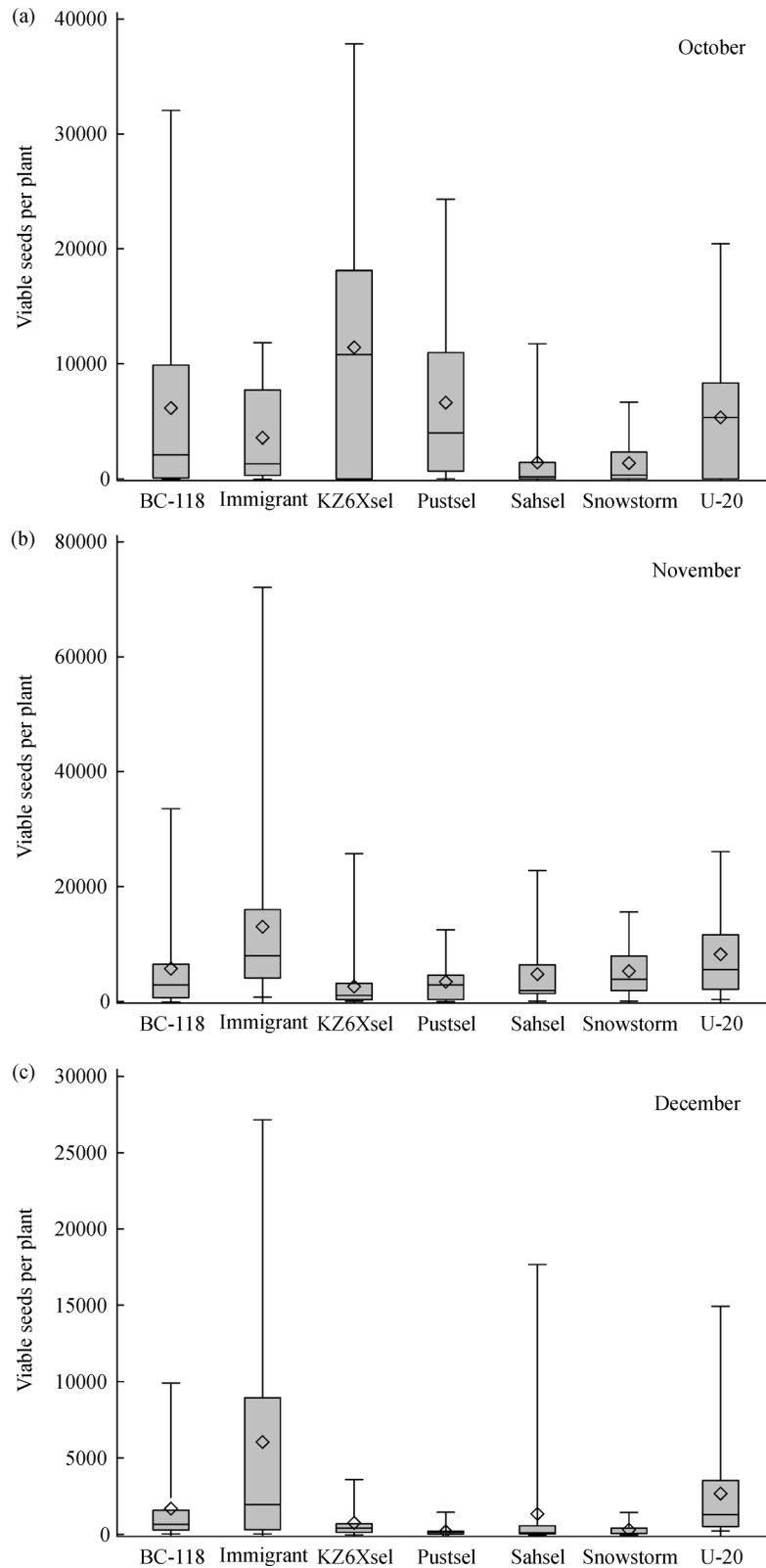


Fig. 4 Skeletal boxplot graphs of the variability in viable seed yield (seeds per plant) among individual plants of seven forage kochia accessions harvested in the first week of October (a), November (b), and December (c) in 2009, 2010, and 2011, respectively. Whiskers represent the extreme range of values. The top and bottom of each box is the 75th and 25th percentile, respectively, and diamonds and lines represent the mean and median, respectively.

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Compliance with ethics guidelines Cody F. Creech, Blair L. Waldron, Corey V. Ransom, Dale R. ZoBell, and Joseph Earl Creech declare they have no conflicts of interest or financial conflicts to disclose.

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