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# Salt Tolerance of Oilseed Crops during Establishment

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**Abstract:** Bioenergy production in arid and semi-arid regions is viewed as being limited due to water resource constraints and potential competition with food production. However, there are crop rotation niches as well as opportunities for utilizing saline soils and water which are not conducive to high value crop production. An exploratory study was thus conducted in a greenhouse for assessing salt tolerance of various oilseed crops during establishment, which is often the critical stage for successful production. Canola (*Brassica napus*), and safflower (*Carthamus tinctorius*) were salt-tolerant during germination, but emergence was curtailed owing to soil crusting. *Camelina* (*C. sativa*) germinated well, yet emergence was poor, probably due to weak hypocotyl. Field pennycress (*Thlaspi arvense*) and *Lesquerella (L. fendleri*) were salt sensitive, and could not germinate in NaCl solutions higher than 50 mM. *Salicornia* (*S. bigelovii*), a halophyte, is extremely salt-tolerant once established, but not during seedling emergence. Seedling emergence which had little correlation with salt tolerance of established plants, was constrained not only by salt tolerance at germination and the pattern of salt accumulation at the soil surface, but also by seed size and soil crust development. At the present state of field management capability, safflower and canola are among the most promising oilseed crops for saline areas, and can potentially be grown as a winter rotation crop with a comparatively low water requirement. For the species with small seed, crop improvements towards greater seedling vigor as well as the effective establishment methods have to be developed.

**Key Words:** Bioenergy, Oilseed, Saline water, Salt tolerance

## 1. Introduction

Increasing concern over energy supply, especially of transportation fuel, the carbon balance, as well as the increasing need to better manage saline soils and water has generated an interest in salt tolerant species which can be used as feedstock or fuelwood. Oilseed crops, especially canola and safflower are among the crops which can readily produce transportation fuel. In the American West, these crops fit in the rotation niches as winter crops, and their water requirement is comparatively low. These crops are also known to be salt-tolerant once established (**Table 1**). Soil salinity which may cause a 15% yield reduction of canola is 12 dS $m^{-1}$ in the saturation extract (Francois, 1994), and that for safflower, 9.3 dS $m^{-1}$ (Francois and Bernstein, 1964). These values can be compared against that for cotton (11 dS $m^{-1}$), which is among the most salt-tolerant crops commercially grown. *Camelina* with a low fertility requirement and a short growing season may have potential for biodiesel production (e.g., Gugel and Falk, 2006). Field pennycress has high contents of erucic and linoleic acids, and grows wild in Northern states and Canada (Moser, 2009). *Lesquerella* (*L. fendleri*) is a potential crop to produce engine lubricants (Adam *et al*., 2007), and unlike castor (*Ricinus communis*), it does not contain toxic substances. Salt tolerance of established *Lesquerella* is rated “moderately tolerant,” 6.9 dS $m^{-1}$ (Grieve *et al*., 1997). Salt tolerance of *Camelina* and pennycress is unknown. Salicornia is a halophyte native to estuaries, and was grown with seawater at a 30% leaching fraction in lysimeter (Glenn *et al*., 1997). It is among a few halophytes which can be grown with highly saline or sea water (Glenn *et al*., 1997; Grattan, *et al*., 2008). The seed of this plant is very small (<0.05 g/100 seed), and its salt tolerance during seedling establishment is unknown.

Difficulties of establishing field crops in saline soils were pointed out years ago (e.g., Bernstein and Fireman, 1957). The primary cause of poor emergence was attributed to salt accumulation in crop beds where seed is usually placed. Difficulties of establishing certain vegetable crops with water of elevated salinity were shown to be hypocotyl mortality by the salts deposited at the soil surface (Miyamoto *et al*., 1985) or seedling kills by salts splashed during passing showers, but not due to poor germination (Miyamoto *et al*., 1986). Field experience in far West Texas has shown difficulties with establishing oilseed crops using water having the electrical conductivity (EC) of 4 to 5 dS $m^{-1}$ (personal communication). The primary objective of this study was to examine salt tolerance of promising oilseed crops during germination and emergence. Another objective was to explore strategies for improving seedling establishment.

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**Table 1. The oilseed crops tested.**

| Common Name | Scientific Name | ECe[1] 15% | Cultivars tested for this study [2] |
|---|---|---|---|
| Canola (*Brassica napus*) | | 11.9 | DKW 45-10, 47-15 |
| Safflower (*Carthamus tinctorious*) | | 9.3 | PI 485984, 406002 CW99OL |
| Camelina (*C. sativa*) | | ? | BSX-WG1, WG3 |
| Pennycress (*T. arvense*) | | ? | Patton, USDA W12 |
| Lesquerella (*L. fendleri*) | | 6.9 | USDA, A3219, 01L0 |
| Salicornia (*S. bigelovii*) | | >30.0 | U of A selection |

[1] Salinity of the soil saturation extract which causes a 15% reduction in growth or yields.
[2] DKW, Dekalab; PI, USDA lines; CW, Cal West, BSK, Bluesun;

## 2. Material and Methods

### 2.1. Germination experiment

Seed germination of cultivars shown in Table 1 was observed in petri-dishes containing various saline solutions. The temperature of the incubator was 23 ± 2°C, except for *Salicornia* which required a higher temperature of 8 °C (Rivers and Weber, 1971). The saline solutions were prepared by adding NaCl to tap water (0.8 dS $m^{-1}$) at three rates, 100, 200, and 300 me $L^{-1}$. In addition, 100 me $L^{-1}$ of $CaCl_2$ and $Na_2SO_4$ were added for assessing salt type effects. For *Lesquerella* and pennycress only, 50 me $L^{-1}$ saline solutions were added. Sterilized glass petri-dishes with a sheet of a filter paper contained 5 ml of each solution which was changed every other day. Seed germination was observed in triplicates using 50 seeds per dish. The seed was considered germinated when the length of radicle exceeded that of the seed. The length of hypocotyl was also measured using five randomly selected samples, after the incubation period of 5 to 7 days.

### 2.2. Seedling emergence and mortality experiments

A preliminary experiment was conducted in a greenhouse which was regulated at 15°C at night and 30°C during day hours. It was for determining appropriate seeding depths under two watering methods; one subirrigated by placing potted soils in a shallow pan of water, and another irrigated from the top. Three soil types were used; Bluepoint loamy sand, Harkey silt loam, and Glendale silty clay (Typic Torrifluvents). The electrical conductivity of the soil saturation extract ($EC_e$) of these soil samples were 0.8, 2.2 and 1.2 dS $m^{-1}$, respectively. Soil samples (<4 mm) were placed in greenhouse pots (15 cm in diameter and depth). Seed (20 each per pot) was placed on a smoothed soil surface, and was covered with the soil which provides a thickness of 0.25, 0.5, 1.0, and 2.0 cm. The entire greenhouse was covered with a shade cloth which provides 70% light transmission. The pan evaporation in the greenhouse averaged 7 mm/day or the potential evaporation rate of 5 mm/day. Irrigation water was applied once a week at 4.5 cm per application. Seedlings that emerged were counted daily or bi-daily.

Subsequently, two experiments were conducted. The first one utilized Harkey silt loam with three levels of soil salinity (1.2, 3.8 and 6.8 dS $m^{-1}$), and tap water for irrigation. The second experiment used three saline solutions with the EC of 4.1, 8.2 and 12.1 dS $m^{-1}$. The saline water was prepared by adding NaCl, $CaCl_2$ and $Na_2SO_4$ to tap water at the chemical equivalent ratio of 2:1:1 to yield the total salt concentration of 40, 80 and 120 me $L^{-1}$. All other procedures used were the same as those used for the preliminary experiment, except for irrigation. The irrigation frequency was increased to twice a week, but using the same amount of water per week, except for *Salicornia* which was watered daily. Seedlings which have emerged as well as experienced mortality were recorded.

One week to 10 days after emergence, seedlings were sprayed with NaCl solutions having concentrations of 100, 200, 300 and 400 mM, until completely wet. The sprayed seedlings were placed in humidity and temperature controlled glass chamber (85% humidity at 22°C). Seedling mortality was then observed for a week, and this process was repeated for another week.

The analysis of variance (ANOVA) was performed for germination and seedling emergence data using a split plot design (Steele and Torrier, 1960). The mean separation was performed at a 5% level.

### 2.3 Soil Salinity and Strength Measurements

Additional potted soils were prepared for measuring soil salinity and strength. These pots were irrigated with the saline solutions mentioned above, one set under subirrigation, and another set top-irrigated. Soil strength was measured in four replicates on 6th day into the experiment with a soil penetrometer. The potted soils were then sectioned to 0-0.5, 0.5-1, 1-3, and 3-5 cm. The samples in duplicate were analyzed for salinity of the saturation extract (Rhoades and Miyamoto, 1990).

## 3. Results

### 3.1. Germination

The final germination counts as well as the days required to obtain 80% of the final counts are shown in **Table 2**. Because of the space limitation, only the cultivar which provided the best germination are shown. Both canola and safflower germinated in 2 to 4 days when incubated in tap water and in 100 mM solutions. In 200 mM solution, seed germination slowed (4 to 5 days), and the final germination percentage was reduced to 40 to 50%. No germination occurred at 300 mM. *Camelina* also germinated in 2 days, but the germination in

**Table 2. The final germination percentage, and the days required to obtain 80% of the final germination.**

| Crops | Treatments | Tap | NaCl | | | $CaCl_2$ | $Na_2SO_4$ |
|---|---|---|---|---|---|---|---|
| | Conc. (me $L^{-1}$) | 8 | 50 | 100 | 200 | 100 | 100 |
| **Canola (DKW 45-10)** | | | | | | | |
| | Germ. (%) | 70a | - | 57a[2] | 47b | 60a | 63a |
| | Days[1] | 2.7 | - | 2.8 | 3.9 | 3.2 | 2.8 |
| **Safflower (485984)** | | | | | | | |
| | Germ. (%) | 82a | - | 63b | 39c | 65b | 62b |
| | Days | 4 | - | 4.1 | 4.9 | 5.1 | 4.5 |
| **Camelina (BSX-WG1)** | | | | | | | |
| | Germ. (%) | 88a | - | 81a | 23c | 75b | 73b |
| | Days | 1.9 | - | 2 | 5.8 | 2.4 | 2 |
| **Pennycress (Patton)** | | | | | | | |
| | Germ. (%) | 100a | 68b | 5e | 0 | 40c | 30d |
| | Days | 3 | 4.5 | - | - | 8 | 9 |
| **Lesquerella (01L0)** | | | | | | (50)[3] | (50)[3] |
| | Germ. (%) | 58a | 30c | 11d | 0 | 46b | 32c |
| | Days | 4 | 4 | 5 | - | 7 | 7 |
| | | | | NaCl (me $L^{-1}$) | | | |
| **Salicornia (U of A)** | | | | 300 | 400 | 500 | 600 |
| | Germ. (%) | 90a | - | 80b | 70c | 40d | 28e |
| | Days | 5 | - | 6.4 | 6.6 | 7.2 | 7.8 |

[1] Days required for attaining 80% of the final germination counts

[2] The numbers in columns followed by the same letter are not significantly different

[3] The numbers in parenthesis are concentrations of $CaCl_2$ and $Na_2SO_4$

200 mM solution was reduced to 23%. Pennycress germinated in 50 mM NaCl solution, but little in 100 mM solutions. It germinated better in $CaCl_2$ or $Na_2SO_4$ solutions than in NaCl solutions at 100 me $L^{-1}$. *Lesquerella* had the germination pattern similar to pennycress. *Salicornia* seed germinated in 5 to 6 days in tap water as well as in 300 mM NaCl solutions. However, germination was reduced and slowed with increasing salinity (Table 2). The germination of cultivars not listed in Table 2 varied, but the difference among the tested cultivars became mostly insignificant when normalized by the germination counts from tap water.

### 3.2. Seedling Emergence and Mortality

The preliminary test conducted has shown that seedling emergence when subirrigated with tap water was largely independent of the seeding depths examined as long as it was within the range specified in **Table 3**. This table also includes the length of hypocotyl measured after 5 to 7 days of incubation in tap water as well as in a 100 me $L^{-1}$ NaCl solution during the germination study. The optimum seeding depth for emergence appeared to be less than the length of hypocotyl, and usually less than 1 cm, but not as deep as 2.0 cm. *Camelina*, pennycress and *Lesquerella* have to be seeded shallow, 0.25 to 0.5 cm, and even shallower for *Salicornia*. The seed size of these species is too small to yield long hypocotyl.

Seedling emergence of canola and safflower from salinized

**Table 3. Seed weight, hypocotyl length and appropriate seedling depth when subirrigated with low salt water.**

| cultivars | Seed weight | Hypocotyl length[1] Tap | NaCl (100) | Optimum Depths |
|---|---|---|---|---|
| | g/ 100 | mm | | |
| Canola (DKW 45-10) | 0.39 | 28 | 8 | 2.5 - 10 |
| Safflower (PI485984) | 2.79 | 32 | 22 | 2.5 - 10 |
| Camelina (BSX-WG1) | 0.10 | 22 | 6 | 2.5 - 5 |
| Pennycress (Patton) | 0.09 | 23 | 2 | 2.5 - 5 |
| Lesquerella (01L0) | 0.07 | 11 | 4 | 2.5 - 5 |
| Salicornia (U of A) | 0.03 | 2 | 2 | 0.0 - 2.5 |

[1] The length of hypocotyl measured after 5 to 7 days of incubation

**Table 4. Seedling emergence from saline soils with seeds placed at three depth, and irrigated with tap water.**

| Crops / Irrigation method | Soil $EC_e$[1] | Days[2] | Emergence: Seeded at 0.25 cm | 0.5 cm | 1.0 cm |
|---|---|---|---|---|---|
| | | | % | | |
| **Canola (**DKW 45-10**)** | | | | | |
| **Sub irrig.** | 1.2 | 4-5 | 60a[2] | 55ab | 55b |
| | 3.8 | 5-6 | 25d | 60a | 70a |
| | 6.4 | 8-7 | 15e | 35c | 40c |
| **Top irrig.** | 1.2 | 4-5 | 45c | 55ab | 50b |
| | 3.8 | 4-5 | 50bc | 50ab | 10d |
| | 6.4 | 4-5 | 60a | 60a | 15d |
| **Safflower (**PI 485984**)** | | | | | |
| **Sub irrig.** | 1.2 | 4-5 | 60ab | 80a | 80a |
| | 3.8 | 5-6 | 65a | 75b | 60c |
| | 6.4 | 6-7 | 55b | 60c | 40d |
| **Top irrig.** | 1.2 | 4-5 | 55b | 60c | 75ab |
| | 3.8 | 5-6 | 55b | 65c | 60c |
| | 6.4 | 6-7 | 65a | 60c | 65c |
| **Camelina (**BSX WG1**)** | | | | | |
| **Sub irrig.** | 1.2 | 5-6 | 20c | 30ab | 0 |
| | 3.8 | 6-7 | 20cd | 30ab | 0 |
| | 6.4 | 7-9 | 10d | 30ab | 0 |
| **Top irrig.** | 1.2 | 5-6 | 35b | 25b | 0 |
| | 3.8 | 6-7 | 25c | 30ab | 0 |
| | 6.4 | 7-9 | 55a | 40a | 0 |

[1] Salinity of the soil saturation extract

[2] The number in columns followed by the same letter are not significantly different

silt loam subirrigated with tap water was significantly lower when seeded at 0.25 cm as compared to seeding at 0.5 or 1.0 cm. When the salinized soils were top-irrigated, however, canola planted at 0.25 cm emerged better than subirrigated case. Seedling emergence of safflower, when subirrigated, was not reduced significantly when initial soil salinity was 3.8 dS $m^{-1}$, but did so at 6.4 dS $m^{-1}$ (**Table 4**). Seedling emergence of canola from salinized silt loam decreased significantly at 3.8 dS $m^{-1}$ when seeded at 0.25 cm, then subirrigated. Seedling emergence of *Camelina* was poor (<30%), regardless of the irrigation methods tested.

Seedling emergence of canola decreased significantly when salinity of irrigation water used increased beyond either 4.1 or 8.2 dS $m^{-1}$, depending on the irrigation methods used (**Table 5**), (Seedling emergence data of canola and safflower at4.1 dS $m^{-1}$ are omitted as they were similar to that from tap water). Top-irrigation suppressed emergence in canola,

**Table 5. Seedling emergence from potted soils irrigated with various saline solutions under two irrigation methods at a seeding depth of 0.5 cm.**

| Crops | Water $EC_w$ ($dS\ m^{-1}$) | Days[1] | Emergence silt loam Sub | silt loam Top | clay loam Sub | clay loam Top |
|---|---|---|---|---|---|---|
| | | | ------ % ------ | | | |
| **Canola** | 0.8 | 4-5 | 65a | 45a | 85a | 40a |
| | 8.2 | 5-6 | 65a | 10c | 55b | 10b |
| | 12.1 | 6-7 | 30b | 0c | 50b | 0b |
| **Safflower** | 0.8 | 4-5 | 85a | 45a | 75a | 50a |
| | 8.2 | 6-7 | 75a | 20b | 75a | 10b |
| | 12.1 | 7-8 | 50b | 0c | 40b | 0b |
| **Camelina** | 0.8 | 5-6 | 30a | 20a | 50a | 10a |
| | 4.1 | 6-7 | 30a | 15a | 40a | 0b |
| | 8.2 | 7-9 | 20b | 0b | 20b | 0b |
| **Pennycress** | 0.8 | 10-12 | 50a | 70a | 75a | 80a |
| | 4.1 | - | 0b | 0b | 0b | 0b |
| **Lesquerella** | 0.8 | 14-20 | 20a | 10a | 20a | 10a |
| | 4.1 | - | 0b | 0b | 0b | 0b |
| **Salicornia** | 0.8 | 18-23 | 0 | 30a | 0 | 10a |
| | 4.1 | 18-23 | 0 | 30a | 0 | 10a |

[1] Days required for attainting 80% of the final emergence under subirrigation
[2] Numbers in column followed by the same letter are not significantly different at a 5% level

safflower and *Camelina*. Seedling emergence from Glendale silty clay was better than from the silt loam, except for *Lesquerella* and *Salicornia* (Table 5).

Seedlings under top-irrigation emerged almost exclusively through soil cracks.

None of the seedlings emerged have died, except for a few seedlings of safflower. Likewise, no seedling mortality was noted when seedlings were sprayed with the saline solutions up to 400 mM.

### 3.3 Salinity Distribution and Soil Strength

Soil salinity measured 6 days into the experiment are shown in **Figure 1**. The data are from Harkey silt loam ($EC_e$ of 2.4 $dS\ m^{-1}$), and those for the silty clay loam were similar, and are not shown. Salt accumulation occurred mostly in the top 0.5 cm.

The soil strength measured on the 6th day averaged 1 and 5 $kg/cm^2$ in sub and top-irrigated silty clay respectively, and 1 and 7 $kg/cm^2$ in silt loam. There was no significant effect of water salinity on soil strength (Table 6).

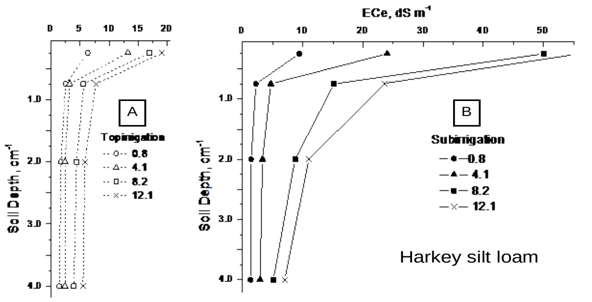


**Fig. 1. Salinity of the saturation extract ($EC_e$) measured 3 days after previous top-irrigation (A) and 6 days after subirrigation (B).**

**Table 6. Soil strength of Harkey salt loam and Glendale silty clay sud or top-irrigated with saline solutions.**

| Water EC $dS\ m^{-1}$ | Soil strength Silt loam sub | Silt loam top | Clay loam sub | Clay loam top |
|---|---|---|---|---|
| | ------ $kg/cm^2$ ------ | | | |
| 0.8 | 1.0a | 6.5b | 1.0a | 4.5b |
| 4.1 | 1.0a | 7.0b | 1.0a | 5.0b |
| 8.2 | 1.0a | 7.0b | 1.0a | 4.5b |

[1] The numbers in row followed by the same letter are not significantly different.

## 4. Discussion

Traditionally, salt tolerance of agricultural crops has been evaluated after plants were established with water of low salinity. This protocol is not always consistent with field practices. In the present study, seedling emergence of canola was reduced at $EC_e$ of 3.8 $dS\ m^{-1}$ (Table 4), while established canola plants were shown to tolerate soil salinity of as high as 11.9 $dS\ m^{-1}$ (Table 1). Likewise, *Lesquerella* failed to establish in soils with ECe of as low as 1.2 $dS\ m^{-1}$ (Table 4), although salt tolerance of established plants was shown to be 6.9 $dS\ m^{-1}$ (Table 1). This conjuncture also applies to *Salicornia*. These findings indicate that salt tolerance of established plants is not a good indicator of seedling establishment in saline areas.

Seedlings of certain plants are known to have salt tolerance which is different from established plants. *Salicornia* seems to fall into this category. The development of succulent tissue did not take place until seedlings had emerged. The slow rate of hypocotyl growth of *Salicornia* can also compound the problem as it allows for a high degree of salt accumulation when subirrigated.

The species tested did not suffer seedling kills, even when sprayed with a 400 mM of NaCl solution. Salt-sensitive species can undergo mortality when sprayed once with saline solutions at the concentration as low as 100 mM (Miyamoto, *et al*., 1986). *Brassica* sp as well as safflower seedlings have the leaves which repel water, a trait desirable for establishment in saline areas.

The salt accumulated at the soil surface might have affected seed germination, especially in pennycress and *Lesquerella*. The seed of these species, especially that of *Lesquerella*, germinated in 50 mM of NaCl, but not at 100 mM with the corresponding EC of 5.5 and 10.5 $dS\ m^{-1}$, respectively. When the seed was placed at 0.25 or 0.5 cm, soil salinity might have been at least 2 to 13 $dS\ m^{-1}$ in ECe when top-irrigated with the saline solution of 4.1 $dS\ m^{-1}$, and 5 to 24 $dS\ m^{-1}$ under subirrigation (Fig. 1). Salinity of soil solutions to which the seed is exposed is 2 to 3 times higher than $EC_e$ (Rhoades and Miyamoto, 1990). Poor seedling emergence observed with pennycress and *Lesquerella* (Table 5) can be attributed to germination failure.

If the germination failure was the only reason for poor emergence, *Camelina* should have provided better seedling emergence than the observed (Table 4), because it is salt-tolerant at germination (Table 2). Likewise, canola should have provided seedling emergence comparable to safflower, as their salt tolerance at germination is comparable (Table 2). The salts accumulated near the soil surface might have reduced hypocotyl growth of canola as well as of *Camelina* and *Lesquerella* (Table 3). This may have lead to reduced emergence.

Seedling emergence of the tested species, except for pennycress and *Salicornia* did not improve through top-irrigation, in spite of the fact that top-irrigation provided lower salinity (Fig. 1). Since seedling emergence under top-irrigated conditions occurred nearly exclusively through soil cracks, it would be realistic to assume that soil crusting was a factor under top-irrigated conditions. In fact, soil strength increased markedly with top-irrigation, especially in Harkey silt loam (**Table 6**). Glendale silty clay contained some organic matter, and registered lower soil strength and in most cases, better emergence than in the silt loam (Tables 5 and 6). The reduction in emergence from top-irrigated conditions was pronounced when saline water was used as opposed to saline soils (Tables 4 and 5). We suspect that salts in the irrigation water had accumulated at the wall of soil cracks, thus limiting emergence through the cracks.

This study indicates that seedling emergence is influenced by many factors, such as seed size which may affect hypocotyl and seedling vigor; irrigation methods which affect vertical distribution of salts as well as soil strength, besides salt tolerance. In oilseed crops having small seed and low salt tolerance at germination, such as pennycress and *Lesquerella*, crop breeding efforts towards improving these traits seem to be prerequisite. In the case of *Salicornia*, a detailed study is needed to clarify the cause(s) of establishment difficulties. Safflower and canola can be established with water of elevated salinity (4 dS $m^{-1}$or somewhat higher), using furrow or corrugation irrigation as long as soil crusting is minimized and the field is leached prior to seeding or with the use of reshaped bed after pre-irrigation. The use of disk-type seeders which plant seeds in V-shaped shallow indentation on the reshaped bed tops may help improve seedling establishment. *Camelina*, which has spindly and thin hypocotyl, has to be seeded shallow, and may be planted using corrugated beds with the V-shaped notches. Under the existing field management capability, safflower and canola appear to be among the promising oilseed crops in saline areas.


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