



Herbicide Resistant Maize Seed Production And Handling

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Cover page photo: Herbicide resistant maize with reduced number of *Striga* plants, and a *Striga* infested maize field.

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Introduction

The objective of this manual is to provide supplementary guidelines to seed companies during maintenance of parental inbred lines, seed production, handling and storage of herbicide resistant maize varieties. This manual should be used as a companion to the Maize Hybrid Seed Production Manual by MacRobert et al. (2014), published by the International Maize and Wheat Improvement Center (CIMMYT). The manual is available at http://repository.cimmyt.org/xmlui/handle/10883/4026?locale-attribute=en. The main hybrid seed production manual contains detailed information on hybrid composition, seed classes, planning seed production requirements, pre-basic, basic, and certified seed production, field management of hybrid seed production, and seed crop budget. Seed companies are strongly advised to consult the manual for detailed information on managing maize hybrid seed production.



The Striga problem and control

Striga spp. are obligate parasitic plants commonly found in cereal-based agricultural systems in sub-Saharan Africa (SSA). Two species of Striga, namely Striga hermonthica (Del.) Benth, and S. asiatica (L.) Kuntze adversely affect grain production of maize and other cereals like millet, sorghum, sugarcane and upland rice in the SSA. Striga produces large amounts of seed that stay dormant in soil for over 20 years until triggered into germination by signals from potential host plants. Striga currently infests up to 40 percent of the cereal production area in SSA, causing substantial annual yield losses in smallholder farmers' fields (Ejeta, 2007). Subsistence farmers in SSA are mostly affected by S. hermonthica and can lose about 20 to 80 percent of their yields due to Striga infestation (Ransom et al., 1990; Haussmann et al., 2000). The problem of Striga can be alleviated partially through crop rotation and intercropping maize with legumes (Oswald and Ransom, 2001), application of organic and inorganic fertilizers (Mumera and Below, 1993), and the use of *Striga* resistant cultivars (Diallo et al., 1997; Menkir et al., 2012). As the efforts to develop *Striga* resistant varieties continue, there is need for stop-gap Striga control options. One such stop-gap Striga control measure is the use of herbicide resistant maize. Maize with herbicide resistance germinates and absorbs some of the herbicide used to coat it. The germinating maize stimulates Striga to germinate and as it attaches to the maize root, it is killed before it can cause any damage. Herbicide that is not absorbed by the maize plant diffuses into the soil and kills Striga seeds that have not germinated. The herbicide therefore decreases the level of *Striga* in the farm through direct attack on the Striga plants and seeds.

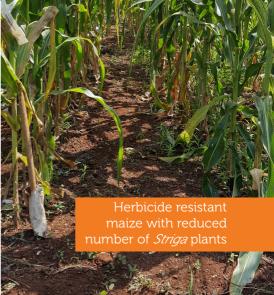
Development of Imidazolinone Resistant (IR) maize for *Striga* control

CIMMYT teamed up with several partners in the private and public sector to develop a seed-based technology for *Striga* control in maize. The technology involves coating of non-transgenic imidazolinone resistant (IR) maize seed with low doses of an acetolactate synthase (ALS) inhibiting herbicide, imazapyr for early *Striga* control before or during attachment to the maize roots (Abayo et al., 1998; Kanampiu et al., 2001). Development of herbicide resistant maize started in 1996 at CIMMYT with incorporation of the IR gene into CIMMYT germplasm using a temperate maize hybrid as the donor of the IR-gene (Kanampiu et al., 2003). Several IR maize varieties have been developed and tested in artificially and naturally *Striga*-infested fields for *Striga* control in eastern and central Africa (Diallo, 2004; Makumbi et al., 2015). Results showed that the use of IR maize varieties reduced the *Striga* seed bank in the soil by over 80 percent in the 30 cm soil top layer, and that imazapyr had no effects on intercropped legumes, when sown at least 12 cm away from the treated maize seed (Kanampiu et al., 2002). Both IR open pollinated varieties (OPVs) and hybrids have been released for commercial production in East Africa.

Maintenance of Imidazolinone Resistant (IR) maize germplasm

The objective of maintaining IR maize germplasm is to ensure that the IR gene is present in all the parents in a homozygous state. The procedures outlined in this manual should be followed to ensure presence of the IR gene in the germplasm.





Maintenance of IR-maize inbred lines

Inbred lines are the parents of the hybrids. Purity of inbred lines should be emphasized and is a priority for seed producers. Imidazolinone resistant maize inbred lines have been developed by CIMMYT and are used to make both hybrids and OPVs. At CIMMYT, the purity of IR lines is maintained by using both standard practices and molecular techniques. Rogueing of off-type plants in nurseries is the basic procedure that should be followed by all seed producers. For IR maize inbred lines, additional steps should be taken to ensure presence of the IR gene. For seed increase, the seed producer has two options to ascertain presence of the IR gene:

- Treat inbred line seed with imazapyr herbicide at the recommended rate. See page 9 and Appendix 1 for guidelines on calculating the right quantity of herbicide to be applied to seed before planting.
- ii. Plant the seed following recommended practices to get good germination. At 4-6 weeks after germination, spray the plants with the recommended dose of the herbicide. The rate applied is 30 g imazapyr a.i. per hectare.

Any of these options will ensure that any heterozygous plants in the nursery will be identified and rogued. At germination, plants of IR maize treated with herbicide will show pale green leaf color but they eventually recover and have the normal green color. Heterozygote plants will develop leaf deformities and show reduced growth rate with herbicide application. Deformities in plants that have been sprayed with herbicide indicate that the gene for IR is in a heterozygote state. DO NOT pollinate or use pollen of plants that show any deformities after herbicide application. Such plants should be removed/rogued from the field before pollination. Selfing should only be done on plants that are completely normal if seed was treated with the herbicide at planting or plants were sprayed with the herbicide.



Maintenance of Imidazolinone resistant open pollinated varieties

Maintenance of IR OPVs is similar to the procedure followed for maize OPVs (see details in Maize Program, 1999). The additional step needed is to ensure presence of the IR gene by coating seed with a herbicide before planting or spraying plants in a nursery with a herbicide after germination (see section on maintenance of IR maize inbred lines).

The maintenance and seed production of OPVs can be managed in three stages of seed multiplication: breeder seed, foundation seed and certified seed. The breeder seed field should show the minimum variation for morphological traits; the certified seed field will have more variation; and the foundation seed field will be intermediate between the two. About 200-300 plants and ears are usually sufficient to represent an OPV and provide adequate breeder seed and other seed classes for maintenance and seed production. Detailed information on OPV maintenance and seed multiplication is found in the manual on development, maintenance, and seed multiplication of OPVs (Maize Program, 1999). Seed producers who grow OPVs are encouraged to consult this publication.



Use of Molecular Markers to screen for homozygosity of IR gene

Molecular markers that can be used to assess homozygosity of the IR gene in maize seed of both inbred lines and OPVs were developed by BASF and are freely available. To use the molecular marker method, 10-15 seeds of an inbred line or 30-50 seeds of an OPV are required for DNA extraction and analysis. The cost of screening each genotype is approximately USD 10. Seed producers interested in using this system can consult CIMMYT for further details. In case results are not conclusive, the seed company is encouraged to spray the crop or treat the seed with the herbicide which will differentiate between homozygous and heterozygous plants.





The general principles of hybrid maize seed production explained in detail in the Maize Hybrid Seed Production Manual (MacRobert et al., 2014) are applicable to IR maize hybrid seed production. In this manual we will explain additional steps to be taken in case of IR maize germplasm.

Making the single cross and final product

Seed harvested from IR maize inbred lines treated with the right amount of herbicide at planting or sprayed with the herbicide before flowering should be pure in terms of IR gene. The seed should be used to make basic (foundation) seed. Seed of both parents should either be treated with the herbicide or sprayed before flowering. Plant the basic seed production field using the recommended pattern for a particular set of parents. The planting pattern will depend on the pollen production characteristics of the male parent and seed yield potential of the female parent. Plants showing deformities and any other off-types should be removed from the field before pollen shed. Harvest single cross seed from plants that did not show any deformities. The single cross seed and corresponding male parent to be used in three-way cross hybrid seed production should be treated with the herbicide. The field should be regularly inspected to remove plants that do not conform to the descriptors as provided by the breeder.



Harvesting seed of IR maize is done according to guidelines given by MacRobert et al. (2014). Seed should be harvested at the right moisture and using appropriate equipment where applicable.

Treatment of seed with the herbicide

Seed should only be treated with the herbicide after thorough cleaning and if ready to either be packaged for sale or for planting. It is important that only the quantity needed for a particular purpose is treated. Facilities and equipment used for treating seed with imazapyr herbicide, transportation, bagging and storage of the treated seed must not be used to handle anv other seed. Use of imazapyr for commercial seed treatment is only possible if the authorities in the respective countries have registered imazapyr herbicide.

Steps required to treat seed with imazapyr herbicide.



Take three samples of 100 seeds from the lot to be treated.

Obtain the weight of each sample of 100 seeds.

Calculate average weight for 100 seed weight

Estimate the weight of one seed.

Determine the quantity of herbicide needed to treat the large sample. Guidelines indicate that 0.57 mg of the herbicide is required to treat one seed.

Example:

- 1. We have 4 kg of seed to be treated.
- 2. Take three samples of 100 seeds each from the 4 kg
- 3. Obtain the weight of each sample of 100 seeds.
 - a. Sample 1...... 30.25g
 - b. Sample 2...... 30.45g
 - c. Sample 3...... 29.90g
- 4. Calculate average weight for 100 seed weight: (30.25+30.45+29.80)/3 = 30.2 g.
- 5. Estimated weight of one seed = 30.2g/100 = 0.302 g.
- 6. Quantity of herbicide required:
 - a. 4 kg of seed = 4000 g
 - b. 0.302g of the seed requires 0.57mg of herbicide.
 - c. Therefore {(4000*0.57)/0.302} mg of herbicide will be required.
 - = 7,549.7 mg of imazapyr herbicide
 - = 7.55 g of imazapyr herbicide

For large scale commercial treatment of seed, please see Appendix A, which gives guidelines on determining combination of herbicide, water, and insecticide/fungicide required for a given quantity of seed. The general guideline is 30 g of imazapyr per 53,000 plants per hectare.



Storage and transportation of herbicide treated seed

After harvest, seed should be dried, cleaned and stored at the right temperature, moisture and humidity to maintain good germination percentage. The right conditions help prevent growth of fungi on the seed. The seed producer should also ensure that the storage area is free of pests (weevils and/or larger grain borer), which can reduce the quality of seed. Seed of IR maize should NEVER be treated with imazapyr before storage if it is to be stored for an extended period. The herbicide will reduce viability of seed with time. If treated, imazapyr treated seed should be stored in a separate warehouse away from other maize seed. Anyone handling herbicide treated seed should use gloves at all times. Washing hands after working with herbicide treated seed is important for safety. Seed companies and stockists dealing with IR maize seed should hand out gloves to farmers. It is advisable to attach a pair of gloves on each seed packet.



General guidelines

- Store seed treatment herbicide only in closed original containers.
- Store the herbicide in a separate room away from seed and other seed treatment products.
- Do not store herbicide treated seed for more than one year.
- Do not transport herbicide treated seed together with other seed.
- Properly wash/clean any loading space where herbicide treated seed has been placed before using it for other seed.
- Store and handle herbicide treated seed in a separate warehouse, separate from other seed.
- Facilities and equipment used for the treatment of seed with herbicide, for transportation, bagging and storage of treated seed must not be used to handle any other seed.
- Do not store open herbicide treated seed bags.
- Hand out the "Farmer's Stewardship Protocol" with each herbicide treated seed bag.
- Wash hands after handling herbicide treated seed bags and before touching any other seed.
- Only allow trained personnel to handle and sell herbicide treated seed.





Guidelines for seed stockists

- Only buy the amount of herbicide treated seed you are sure to sell in the crop season.
- Only buy and sell herbicide treated seed bags that are properly sealed and marked with the StrigAway® Production System logo.
- Do not store herbicide treated seed for more than one year.
- Do not transport herbicide treated seed together with other seed.
- Properly wash/clean any loading space where herbicide treated seed has been transported before transporting other seed.
- Store and handle herbicide treated seed in a separate warehouse, away from other seed.
- Do not store open herbicide treated seed bags.
- Hand out "Farmer's Stewardship Protocol" with each herbicide treated seed bag.
- Wash hands after handling herbicide treated seed bags and before touching any other seed.
- Only allow trained personnel to handle and sell herbicide treated seed.



Guidelines for farmers

- Only buy herbicide treated seed from an authorized distributor or retailer.
- Buy new herbicide treated seed every year. Saved or recycled seed does not control Striga.
- Make sure you buy herbicide treated seed bags that are properly sealed and marked with the StrigAway® logo.
- Only buy the quantity of herbicide treated seed needed for the area to be planted – avoid seed carry over.
- Do not store the herbicide treated seed for more than one year.
- Do not store open bags of seed.
- Store the herbicide treated seed separate from any other seed.
- Use gloves when planting herbicide treated seed.
- Intercropping maize with other crops is possible with herbicide treated maize; however, a minimum distance of 15 cm between the maize plant and the second crop is required.
- Wash hands and use new gloves after planting or handling herbicide treated seed and before touching any other seed.
- Don't feed animals herbicide treated seed or eat animals that have been fed with herbicide treated seed.

Land preparation and planting treated seed

Imazapyr herbicide will kill any ordinary maize without herbicide resistance. So it is very important to avoid handling IR maize seed and then non-IR maize seed.



Land preparation and planting guidelines

- Land preparation should be well done and the field should have good drainage.
- Avoid planting in areas with depressions as this causes water logging in such areas and hence poor germination.
- Planting should be done only when there is enough soil
 moisture and with proper rains. Avoid planting immediately
 with first shower because this is normally followed by a
 dry spell. This can cause poor emergence and poor stand
 establishment.
- The personnel who plant the herbicide treated maize seed should not be the ones to plant maize seed not treated with the herbicide. If the same person has to do it, then he/she should wash their hands with soap before handling seed.
- Any person planting herbicide treated maize seed should wear gloves to avoid direct contact with the herbicide coated seed.

Source of Imidazolinone Resistant (IR) maize germplasm

Imidazolinone resistant (IR) maize germplasm is freely available from CIMMYT. This germplasm is either parental inbred lines to be used for breeding and production of IR maize hybrids or OPVs. Final products (hybrids and OPVs) are available from CIMMYT for commercialization upon signing a licensing agreement.

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Appendix A1

Seed Treatment Protocol

- 1. Weigh three samples of 100 maize seeds per seed lot (max.10) to determine average seed weight. The amount of *StrigAway* used is dependent on seed size.
 - a) Sample 1 = 32.82 g
 - b) Sample 2 = 32.94 g
 - c) Sample 3 = 32.82 g
 - d) Average 100 seed weight = 32.86 g or 0.03286 kg
- 2. Average planting density of the maize seed, based on spacing of 75 x 50 cm and 2 seeds per hill = 53,000 seeds per hectare.
- 3. Determine seed weight that will be planted per hectare:

A = (0.03286 kg per 100 seeds x 53,000 seeds per hectare) / 100 = 17.5 kg of seeds to plant per hectare

4. Target use rates per hectare are as follows:

StrigAway: 30 grams/ha Murtano: 50 grams/ha Water: 350 mL/ha

- 5. To create a herbicide seed treatment slurry for one ton (= 1,000 kg) of seed, combine the following ingredients in a seed treater:
 - a. 350 mL of water x 1000/17.5 kg per ha
 - b. 50 grams of Murtano x 1000/17.5 kg per ha
 - c. 30.4 grams of imazapyr (98% technical grade) x 1000/17.5 kg per ha (To create the slurry for seed quantities greater than one ton, please consult the Table below.)
- Operate stirrer in the mixing tank gently, until components are completely dissolved, forming a homogeneous slurry.
- Calibrate seed treater to deliver the correct amount of slurry per ton of seed.
 As the amount of slurry will vary by seed size, please consult the Table below to determine the proper amount of slurry per amount of seed.
- 8. During application, repeatedly check amounts of seed and amounts of slurry consumed. Readjust machinery settings if actual slurry consumption deviates from target rate.
- 9. Operate seed treater in a way that a uniform coating of the slurry forms around the seed and that all seeds carry equal amounts of seed coating.

	10 tons (10,000Kg)		10 tons	9 tons (9,000Kg)			8 tons (8,000Kg)				(7,000Kg)	7 tons		(6,000Kg)	6 tons		(5,000Kg)	5 tons		(4,000Kg)	4 tons		(3,000Kg)	3 tons		(2,000Kg)	2 tons		(1,000kgs)	1 ton	Amount of seed to be treated	
origaway (kg)		Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Slurry ingredient	
19.12		31.45	220.10	17.21	28.30	198.10	15.30	25.16	176.10	13.38	22.01	154.10	11.47	18.87	132.10	9.56	15.72	110.10	7.65	12.58	88.10	5.74	9.43	66.00	3.82	6.29	44.00	1.91	3.14	22.00	30.00	Ī
Ια.αΙ		30.93	216.50	16.93	27.84	194.90	15.04	24.74	173.20	13.16	21.65	151.60	11.28	18.56	129.90	9.40	15.47	108.30	7.52	12.37	86.60	5.64	9.28	65.00	3.76	6.19	43.30	1.88	3.09	21.70	30.50	Ť
10.50		30.43	213.00	16.65	27.39	191.70	14.80	24.35	170.40	12.95	21.30	149.10	11.10	18.26	127.80	9.25	15.22	106.50	7.40	12.17	85.20	5.55	9.13	63.90	3.70	6.09	42.60	1.85	3.04	21.30	31.00	
10.21		29.95	209.60	16.39	26.95	188.70	14.57	23.96	167.70	12.75	20.96	146.80	10.93	17.97	125.80	9.10	14.97	104.80	7.28	11.98	83.90	5.46	8.98	62.90	3.64	5.99	41.90	1.82	2.99	21.00	31.50	Ī
17.92	1	29.48	206.40	16.13	26.53	185.70	14.34	23.58	165.10	12.55	20.64	144.50	10.75	17.69	123.80	8.96	14.74	103.20	7.17	11.79	82.50	5.38	8.84	61.90	3.58	5.90	41.30	1.79	2.95	20.60	32.00	
17.00	1	29.03	203.20	15.88	26.12	182.90	14.12	23.22	162.60	12.35	20.32	142.20	10.59	17.42	121.90	8.82	14.51	101.60	7.06	11.61	81.30	5.29	8.71	61.00	3.53	5.81	40.60	1.76	2.90	20.30	32.50	-
17.38	1	28.59	200.10	15.64	25.73	180.10	13.91	22.87	160.10	12.17	20.01	140.10	10.43	17.15	120.10	8.69	14.29	100.10	6.95	11.44	80.00	5.21	8.58	60.00	3.48	5.72	40.00	1.74	2.86	20.00	33.00	Aver
17.12	1	28.16	197.10	15.41	25.34	177.40	13.70	22.53	157.70	11.99	19.71	138.00	10.27	16.90	118.30	8.56	14.08	98.60	6.85	11.26	78.90	5.14	8.45	59.10	3.42	5.63	39.40	1.71	2.82	19.70	33.50	Average 100 seed weight (grams)
10.07	1	27.75	194.20	15.18	24.97	174.80	13.50	22.20	155.40	11.81	19.42	136.00	10.12	16.65	116.50	8.44	13.87	97.10	6.75	11.10	77.70	5.06	8.32	58.30	3.37	5.55	38.80	1.69	2.77	19.40	34.00	seed wei
16.63		27.34	191.40	14.96	24.61	172.30	13.30	21.88	153.10	11.64	19.14	134.00	9.98	16.41	114.80	8.31	13.67	95.70	6.65	10.94	76.60	4.99	8.20	57.40	3.33	5.47	38.30	1.66	2.73	19.10	34.50	ght (gran
10.39		26.95	188.70	14.75	24.26	169.80	13.11	21.56	150.90	11.47	18.87	132.10	9.83	16.17	113.20	8.19	13.48	94.30	6.56	10.78	75.50	4.92	8.09	56.60	3.28	5.39	37.70	1.64	2.70	18.90	35.00	is)
10.10		26.57	186.00	14.54	23.92	167.40	12.93	21.26	148.80	11.31	18.60	130.20	9.69	15.94	111.60	8.08	13.29	93.00	6.46	10.63	74.40	4.85	7.97	55.80	3.23	5.31	37.20	1.62	2.66	18.60	35.50	
15.93	0 1	26.21	183.40	14.34	23.58	165.10	12.75	20.96	146.80	11.15	18.34	128.40	9.56	15.72	110.10	7.97	13.10	91.70	6.37	10.48	73.40	4.78	7.86	55.00	3.19	5.24	36.70	1.59	2.62	18.30	36.00	
15.71		25.85	180.90	14.14	23.26	162.80	12.57	20.68	144.70	11.00	18.09	126.60	9.43	15.51	108.60	7.86	12.92	90.50	6.29	10.34	72.40	4.71	7.75	54.30	3.14	5.17	36.20	1.57	2.58	18.10	36.50	Ī
10.50	1	25.50	178.50	13.95	22.95	160.60	12.40	20.40	142.80	10.85	17.85	124.90	9.30	15.30	107.10	7.75	12.75	89.20	6.20	10.20	71.40	4.65	7.65	53.50	3.10	5.10	35.70	1.55	2.55	17.80	37.00	Ī
15.30		25.16	176.10	13.77	22.64	158.50	12.24	20.13	140.90	10.71	17.61	123.30	9.18	15.09	105.70	7.65	12.58	88.10	6.12	10.06	70.40	4.59	7.55	52.80	3.06	5.03	35.20	1.53	2.52	17.60	37.50	
15.09		24.83	173.80	13.58	22.34	156.40	12.08	19.86	139.00	10.57	17.38	121.60	9.06	14.90	104.30	7.55	12.41	86.90	6.04	9.93	69.50	4.53	7.45	52.10	3.02	4.97	34.80	1.51	2.48	17.40	38.00	-
14.90		24.50	171.50	13.41	22.05	154.40	11.92	19.60	137.20	10.43	17.15	120.10	8.94	14.70	102.90	7.45	12.25	85.80	5.96	9.80	68.60	4.47	7.35	51.50	2.98	4.90	34.30	1.49	2.45	17.20	38.50	
14./1		24.19	169.30	13.24	21.77	152.40	11.77	19.35	135.50	10.30	16.93	118.50	8.82	14.51	101.60	7.35	12.09	84.70	5.88	9.68	67.70	4.41	7.26	50.80	2.94	4.84	33.90	1.47	2.42	16.90	39.00	
14.52		23.88	167.20	13.07	21.50	150.50	11.62	19.11	133.70	10.16	16.72	117.00	8.71	14.33	100.30	7.26	11.94	83.60	5.81	9.55	66.90	4.36	7.17	50.20	2.90	4.78	33.40	1.45	2.39	16.70	39.50	

Table adapted from guidelines developed by BASF

	10 tons (10,000Kg)			9 tons (9,000Kg)			8 tons (8,000Kg)			(7,000Kg)	7 tons		(6,000Kg)	6 tons		(5,000Kg)	5 tons		(4,000Kg)	4 tons		(3,000Kg)	3 tons		(2,000Kg)	2 tons		(1,000kgs)	1 ton	Amount of seed to be treated	
Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Strigaway (Kg)	Murtano (Kg)	Water (L)	Slurry ingredient	
28.68	47.17	330.20	25.81	42.45	297.20	22.94	37.74	264.20	20.08	33.02	231.10	17.21	28.30	198.10	14.34	23.58	165.10	11.47	18.87	132.10	8.60	14.15	99.10	5.74	9.43	66.00	2.87	4.72	33.00	20.00	
27.98	46.02	322.10	25.18	41.42	289.90	22.38	36.82	257.70	19.59	32.21	225.50	16.79	27.61	193.30	13.99	23.01	161.10	11.19	18.41	128.90	8.39	13.81	96.60	5.60	9.20	64.40	2.80	4.60	32.20	20.50	
27.31	44.92	314.50	24.58	40.43	283.00	21.85	35.94	251.60	19.12	31.45	220.10	16.39	26.95	188.70	13.66	22.46	157.20	10.93	17.97	125.80	8.19	13.48	94.30	5.46	8.98	62.90	2.73	4.49	31.40	21.00	
26.68	43.88	307.20	24.01	39.49	276.40	21.34	35.10	245.70	18.67	30.72	215.00	16.01	26.33	184.30	13.34	21.94	153.60	10.67	17.55	122.90	8.00	13.16	92.10	5.34	8.78	61.40	2.67	4.39	30.70	21.50	
26.07	42.88	300.20	23.46	38.59	270.20	20.86	34.31	240.10	18.25	30.02	210.10	15.64	25.73	180.10	13.04	21.44	150.10	10.43	17.15	120.10	7.82	12.86	90.10	5.21	8.58	60.00	2.61	4.29	30.00	22.00	
25.49	41.93	293.50	22.94	37.74	264.20	20.39	33.54	234.80	17.84	29.35	205.50	15.30	25.16	176.10	12.75	20.96	146.80	10.20	16.77	117.40	7.65	12.58	88.10	5.10	8.39	58.70	2.55	4.19	29.40	22.50	
24.94	41.02	287.10	22.44	36.92	258.40	19.95	32.81	229.70	17.46	28.71	201.00	14.96	24.61	172.30	12.47	20.51	143.60	9.98	16.41	114.80	7.48	12.31	86.10	4.99	8.20	57.40	2.49	4.10	28.70	23.00	Ave
24.41	40.14	281.00	21.97	36.13	252.90	19.53	32.12	224.80	17.09	28.10	196.70	14.64	24.09	168.60	12.20	20.07	140.50	9.76	16.06	112.40	7.32	12.04	84.30	4.88	8.03	56.20	2.44	4.01	28.10	23.50	Average 100 seed weight (grams)
23.90	39.31	275.20	21.51	35.38	247.60	19.12	31.45	220.10	16.73	27.52	192.60	14.34	23.58	165.10	11.95	19.65	137.60	9.56	15.72	110.10	7.17	11.79	82.50	4.78	7.86	55.00	2.39	3.93	27.50	24.00	seed we
23.41	38.51	269.50	21.07	34.66	242.60	18.73	30.80	215.60	16.39	26.95	188.70	14.05	23.10	161.70	11.71	19.25	134.80	9.36	15.40	107.80	7.02	11.55	80.90	4.68	7.70	53.90	2.34	3.85	27.00	24.50	eight (gra
22.94	37.74	264.20	20.65	33.96	237.70	18.35	30.19	211.30	16.06	26.42	184.90	13.77	22.64	158.50	11.47	18.87	132.10	9.18	15.09	105.70	6.88	11.32	79.20	4.59	7.55	52.80	2.29	3.77	26.40	25.00	ms)
22.49	37.00	259.00	20.24	33.30	233.10	17.99	29.60	207.20	15.75	25.90	181.30	13.50	22.20	155.40	11.25	18.50	129.50	9.00	14.80	103.60	6.75	11.10	77.70	4.50	7.40	51.80	2.25	3.70	25.90	25.50	
22.06	36.28	0 254.00	19.85	32.66	0 228.60	17.65	29.03	0 203.20	15.44	25.40	0 177.80	13.24	21.77	0 152.40	11.03	18.14	0 127.00	8.82	14.51	0 101.60	6.62	10.89	76.20	4.41	7.26	50.80	2.21	3.63	25.40	26.00	
21.64	35.60	0 249.20	19.48	32.04	0 224.30	17.32	28.48	0 199.40	15.15	24.92	0 174.40	12.99	21.36	0 149.50	10.82	17.80	0 124.60	8.66	14.24	0 99.70	6.49	10.68	74.80	4.33	7.12	49.80	2.16	3.56	24.90	26.50	
21.24	34.94	0 244.60	19.12	31.45	0 220.10	17.00	27.95	0 195.70	14.87	24.46	0 171.20	12.75	20.96	0 146.80	10.62	17.47	0 122.30	8.50	13.98	97.80	6.37	10.48	73.40	4.25	6.99	48.90	2.12	3.49	24.50	27.00	
20.86	34.31	0 240.10	18.77	30.87	0 216.10	16.69	27.44	0 192.10	14.60	24.01	0 168.10	12.51	20.58	0 144.10	10.43	17.15	0 120.10	8.34	13.72	96.10	6.26	10.29	72.00	4.17	6.86	48.00	2.09	3.43	24.00	27.50	
20.49	33.69	0 235.80	18.44	30.32	0 212.30	16.39	26.95	0 188.70	14.34	23.58	0 165.10	12.29	20.22		10.24	16.85		8.19	13.48	94.30	6.15	10.11	70.80	4.10	6.74	47.20	2.05	3.37	23.60	28.00	
9 20.13	33.10	30 231.70	4 18.11	2 29.79	30 208.50	9 16.10	5 26.48	70 185.40	14.09	3 23.17	10 162.20	9 12.08	2 19.86	141.50 139.00	4 10.06	5 16.55	117.90 115.90	8.05	8 13.24	92.70	6.04	9.93	0 69.50	4.03	6.62) 46.30	2.01	3.31	23.20	28.50	
3 19.78	32.53	70 227.70	17.80	9 29.28	50 204.90	15.82	3 26.02	10 182.20	13.85	7 22.77	20 159.40	3 11.87	19.52	00 136.60	9.89	5 16.27	0 113.90	7.91	13.01	91.10	5.93	9.76	68.30	3.96	6.51) 45.50	1.98	3.25	22.80	29.00	
3 19.44	31.98	70 223.90	17.50	3 28.78	90 201.50	15.55	25.58	20 179.10	5 13.61	7 22.39	10 156.70	7 11.67	2 19.19	30 134.30	9.72	15.99	0 111.90	7.78	12.79	89.50	5.83	9.59	67.20	3.89	6.40) 44.80	1.94	3.20) 22.40) 29.50	

