



Plant Biotechnology

Bayer Russia Plant Biotechnology
Conference

July 2023





Corn Donor Plants and R0 Plant Production in Controlled Environments

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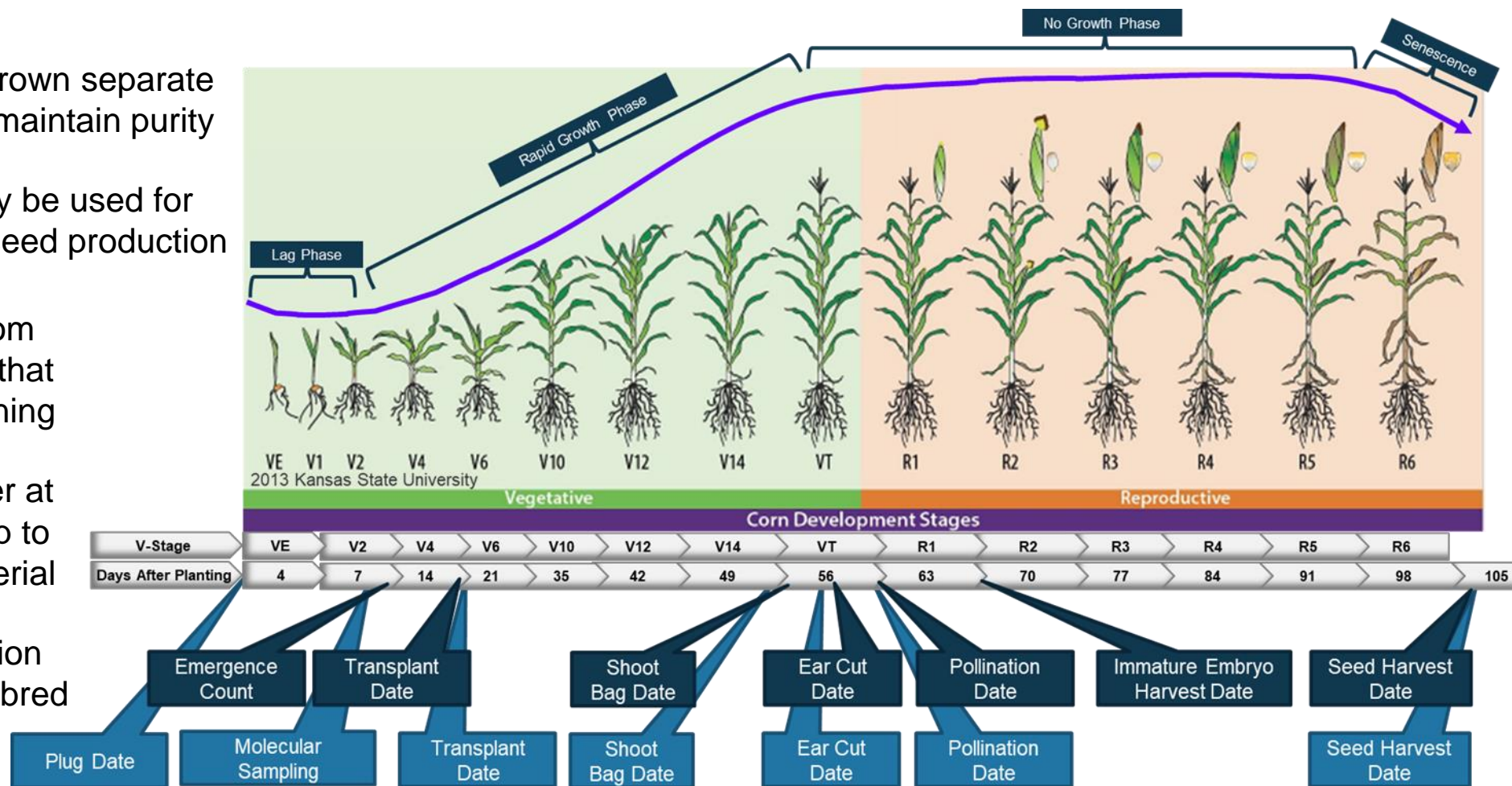
Corn Production in Controlled Environments: **Agenda**

Topic 1	Overview of Corn Production
Topic 2	Corn Donor Plants for Immature Embryo Harvest
Topic 3	Corn Donor Plants for Seed Harvest
Topic 4	Corn R0s Plants for Tissue or Seed Harvest
Topic 5	Summary



Overview of Corn Production: LH244 Germplasm

- Donor corn production from verified seed source
- Donor corn must be grown separate from all other corn to maintain purity
- Donor corn plants may be used for immature embryo or seed production
- R0 corn production from transformed plantlets that pass molecular screening
- R0 plantlets are slower at beginning but catch up to seed propagated material
- R0 population pollination window longer than Inbred population





Environmental Control Parameters: Climate & Fertigation

- During rapid growth phase target even environment and increasing water/nutrients
- Plant growth slows with reproductive development
- As plant senescence (grain fill) occurs, target cooler and drier environment with less water/nutrients

Climate Controls	Vegetative	Reproductive	Range of Control Values
Average Daily Setpoint Temperature	Lower	Higher	26 – 28° C: higher temperatures at higher light levels
Day-Night Temperature Difference	Smaller	Larger	0 – 8° C
Start time for heating in the morning	Earlier	Later	4 hours before sunrise to sunrise
Afternoon temperature increase	None or small	Larger	0 – 2.5° C
Start Time for Day-Night Temperature Decrease	Earlier	Later	2 hours before to 2 hours after sunset
Speed of Day-Night Temperature Decrease	Slower	Faster	0 - 4° C per hour
Humidity deficit	Decrease	Increase	8 - 15 mB
Vapor Pressure Deficit	Lower	Higher	0.8 – 1.5 kPa
CO ₂ enrichment	More	Less	350 – 1500 ppm: Higher CO ₂ at higher light levels

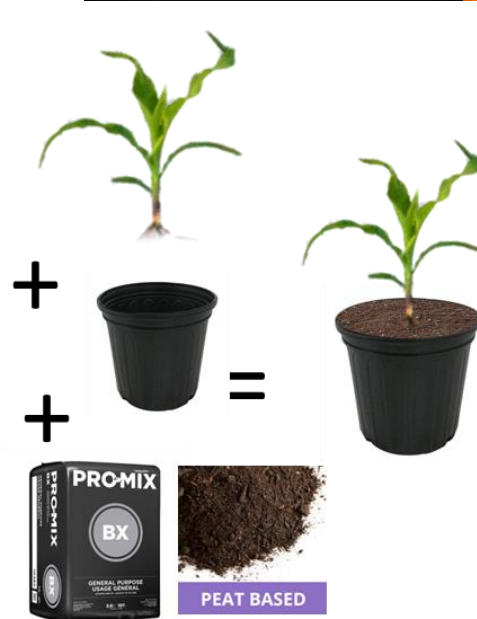
Watering Controls	Vegetative	Reproductive	Range of Control Values
EC Growing Medium	Decrease	Increase	2.8 - 4.5 EC
EC Irrigation Water	Decrease	Increase	2.0 - 2.5 EC
Substrate Water Content	Increase	Decrease	45 - 65%
Day-Night Water Content Decrease	Decrease	Increase	2 - 10% (5 - 15% with Rockwool)
Irrigation cycle length and frequency	Short and Higher	Long and Lower	50 - 150 ml per dripper
Start time first irrigation	Earlier	Later	1 - 3 hours after sunrise/lights on
Stop time last irrigation	Later	Earlier	3 - 5 hours before sunset/lights off



Corn Donor Plant Production: LH244 Germplasm

Items of Special Interest

- All atypical plants should be discarded immediately
- Soil based media as a rooting substrate is undesirable for LH244 production in pots
- LH244 germplasm is highly sensitive to overwatering, especially before V8 stage





Corn Donor Plant Production: Pre-Pollination



Seed planted 3 – 5
days/week and
Germinated in peat
based media

Plugs Ready to
Transplant

Transplanting 1 – 2
times/week

Early Production

Middle Production

29.4/21.1 C (85/70 F) D/N
16 hour photoperiod
No Fertilizer until V2 then
15-5-15 at 2,000 μ S

V3-V4 Transplant Corn
12-16 DAP

Pot filled with peat-
based media
Plant transplanted
Plants watered in with
fertilizer 15-5-15 at
2,000 μ S

29.4/21.1 C (85/70 F) D/N
16 hour photoperiod
Fertilizer 15-5-15 at 2,000 μ S
Wet/Dry cycles until first ear
shoots appear then flush pots



Corn Donor Plant Production: Pre-Pollination

Suggested watering schedule with peat-based media

- 400 ml per minute with spray emitters
- Wet/Dry cycles early then even moisture
- Flush = hand watering with plain water 2 – 3 times to field capacity
- Avoid water stress after flush

Time	Approx. developmental stage	Approx. Irrigation Time	Days
week 1	Germination – V2	Hand water – Plain water	Every 2-3 days
week 2	V3-V4 by day 14	Sub-irrigation or hand water	Every 1-2 days
week 3	Transplant at end of week 2 or start of week 3. V5 by day 18-21.	0:20 - 0:30, two times (8am and 4pm)	Every day (ED)
week 4	V7 by day 28	0:30 - 0:40, two times (8am and 4pm)	Every day (ED)
week 5	V9 by day 35	0:45 - 1 min, two times (8am and 4pm)	Every day (ED)
week 6	V11 by day 42	1 min - 1:15, two times (8am and 4pm)	Every day (ED)
week 7	V14-V15 by day 49. Tassel beginning to emerge. Flush plant when tassel is first felt or visible and increase irrigation so that pots have constant moisture.	2 min, two times (8am and 4pm)	Every day (ED)
week 8	VT and pollen shed by day 56; Possible pollination.	3 min, two times (8am, 4pm)	Every day (ED)
week 9	Pollination (day 56-63)	3 min, two times (8am, 4pm)	Every day (ED)



Corn Donor Plant Production: Pollination



Pollination

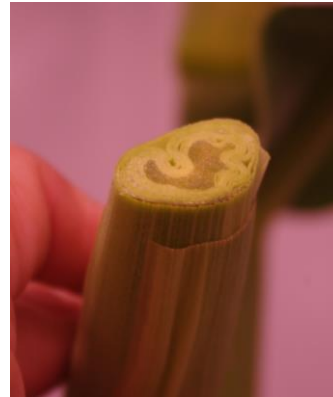
85/70 F (29.4/21.1 C) D/N
16 hour photoperiod
Fertilizer 15-5-15 at 2,000 μ S



Ear Shoot
Bagging



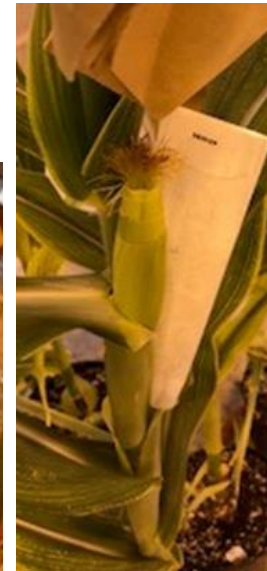
Tassel Set
Up



Ear Cut



Pollen
Collection



Ear Silk
Brush



Pollination

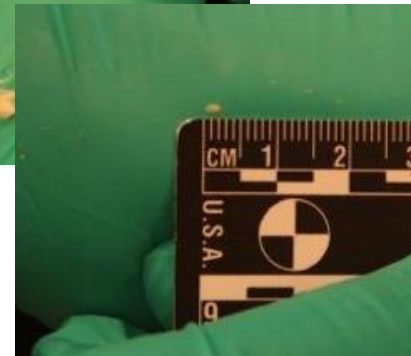
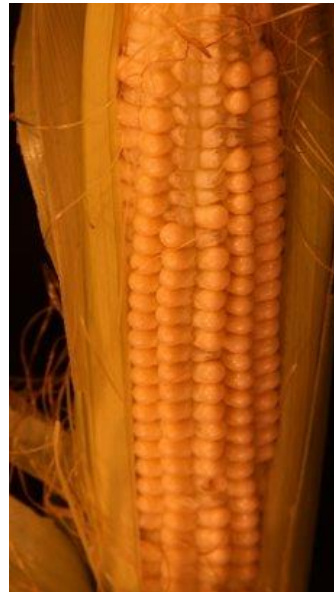


Corn Donor Plant Production: Immature Embryo Path



Immature Embryo
Harvest

85/70 F (29.4/21.1 C) D/N
16 hour photoperiod
Fertilizer 15-5-15 at 2,000 μ S



Peel Back

Embryo
Sizing

Ear Collection

Short-term
Ear storage

Embryo
Harvest



Corn Donor Plant Production: Post-Pollination

Suggested watering schedule with peat-based media

- 400 ml per minute with spray emitters
- Avoid water stress after flush until R4
- Lower water frequency as seed matures to promote drying
- Plants should not exhibit water stress visually until week 14

Time	Approx. developmental stage	Approx. Irrigation Time	Days
week 8	VT and pollen shed by day 56; Possible pollination.	3 min, two times (8am, 4pm)	Every day (ED)
week 9	Pollination (day 56-63)	3 min, two times (8am, 4pm)	Every day (ED)
week 10	Immature embryo harvest 9-11 days after pollination	3 min, two times (8am, 4pm)	Every day (ED)
week 11	Bag lift two weeks after pollination (end of week 11).	3 min, two times (8am, 4pm)	Every day (ED)
week 12	Peel back three weeks after pollination – R4 to R5 (end of week 12 or beginning of week 13).	2 min, two times (8am, 4pm)	Every day (ED)
week 13	Stop water 30 days after pollination (end of week 13).	1 min, two times, then stop.	Every day (ED)
week 14	R6	none	Every day (ED)
week 15	Harvest 42 days after pollination/Dry/ Processing	none	Every day (ED)



Corn Donor Plant Production: Mature Seed Path



Late Production –
Seed Production

85/70 F (29.4/21.1 C) D/N
16 hour photoperiod
Fertilizer 15-5-15 at 2,000 μ S



Bag Lift



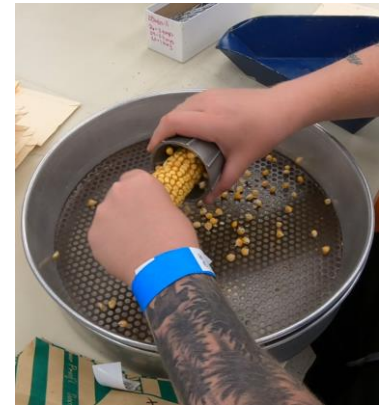
Peel Back



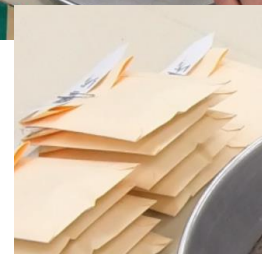
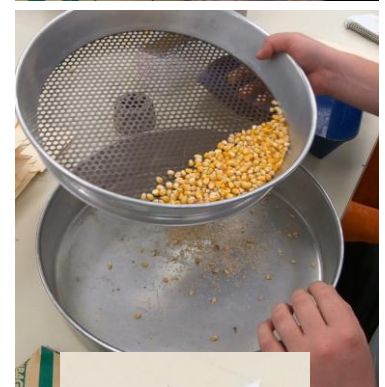
Seed
Maturation



Ear Harvest



Seed
Processing





Corn Donor Plant Summary: LH244 Germplasm

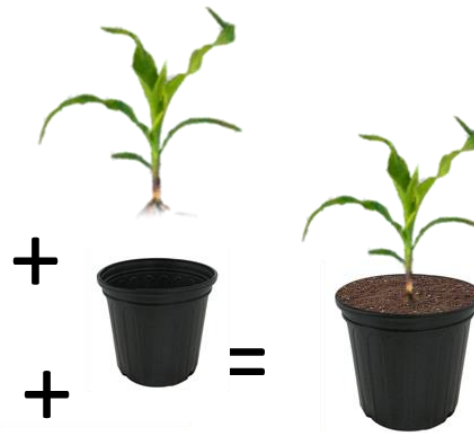
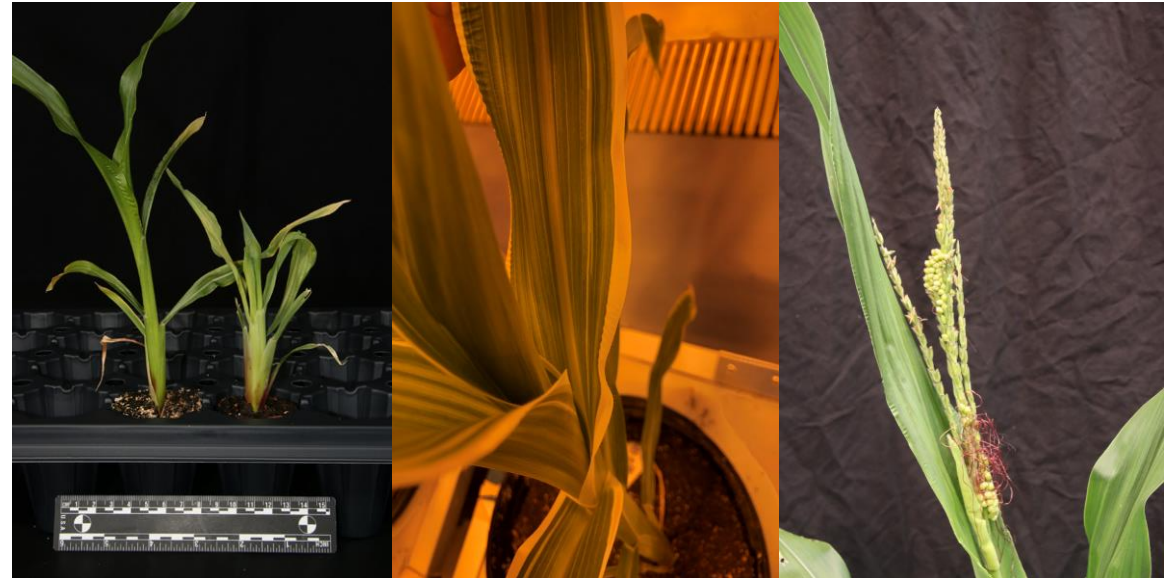
- Sequential plantings provide ears of correct age for immature embryo harvest weekly
- Donor plants should be isolated from all other corn plantings
- Environmental management early in crop cycle impacts later developmental stages
 - Atypical phenotypes should be removed as soon as detected
 - LH244 is extremely sensitive to overwatering during lag and rapid growth phase
 - Slight leaf curl is acceptable until ear shoots are evidenced
 - Water deficit should be avoided once ear shoot are evidenced
- If self pollination is not possible, sib pollinations are acceptable
- Immature embryo harvest occurs approximately 10 days after pollination
- Pollinated ears not used for immature embryo harvest may be advanced to mature seed if needed for future plantings.
 - Must maintain isolation throughout life cycle to ensure LH244 purity



Corn R0 Plant Production: LH244 Germplasm

Items of Special Interest

- All atypical plants should be discarded immediately
 - Off types rarely produce sufficient seed to test in the next generation
 - Rouging should occur before pollination
- LH244 germplasm is highly sensitive to overwatering, especially before V8 stage
- Self pollination is preferred but nicking issues may exist with R0 plants
 - Contingency crosses are possible if female support plants are available





Corn R0 Plant Production: LH244 Germplasm

Items of Special Interest

- Inbred and R0 plants from same genetic background have different phenotypes
 - R0 plants will have more off types than Inbred plants
 - R0 plants often lack vigor
- Self pollination is preferred but nicking issues may exist with R0 plants
 - Tassel and ear health are especially difficult to maintain in R0 plants
 - Ears push late with minimal silk brush
 - Tassels shed early
 - Low quantity and low quality pollen is possible



Inbred

R0

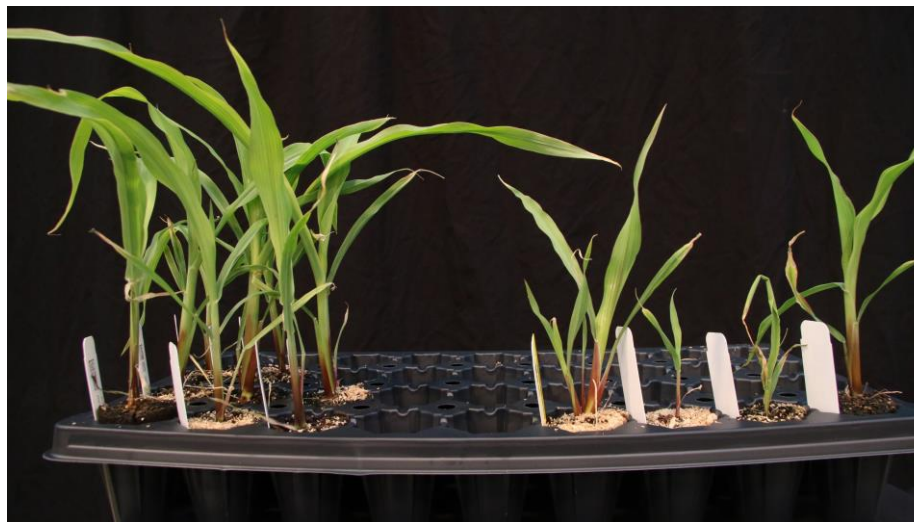
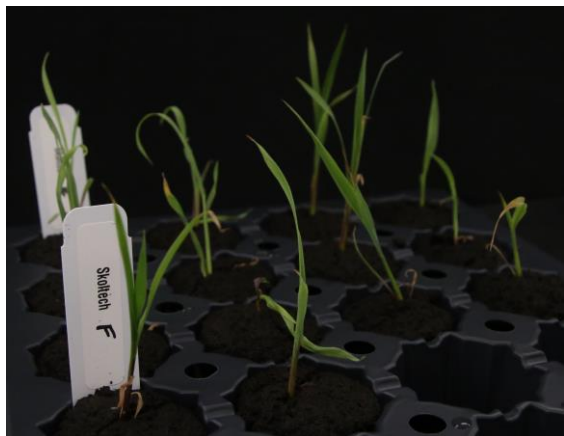


Inbred

R0



Corn R0 Plant Production: Pre-Transplant



Plugs from
Transformation

28.3/22.8 C (83/73 F) D/N
16 hour photoperiod
Fertilizer 15-5-15 + Fe at 2,000 μ S
Optional Shade 30-40%
Mist first 3-5 days

Molecular
Sampling

7-10 DAP

Plugs Ready
to Transplant

V3-V4 Transplant Corn
14-21 DAP

Plug Selection

14-21 DAP

Transplant

Pot filled with peat-based media
Plant transplanted
Plants watered in with
fertilizer 15-5-15 at
2,000 μ S



Corn R0 Plant Production: Pre-Transplant

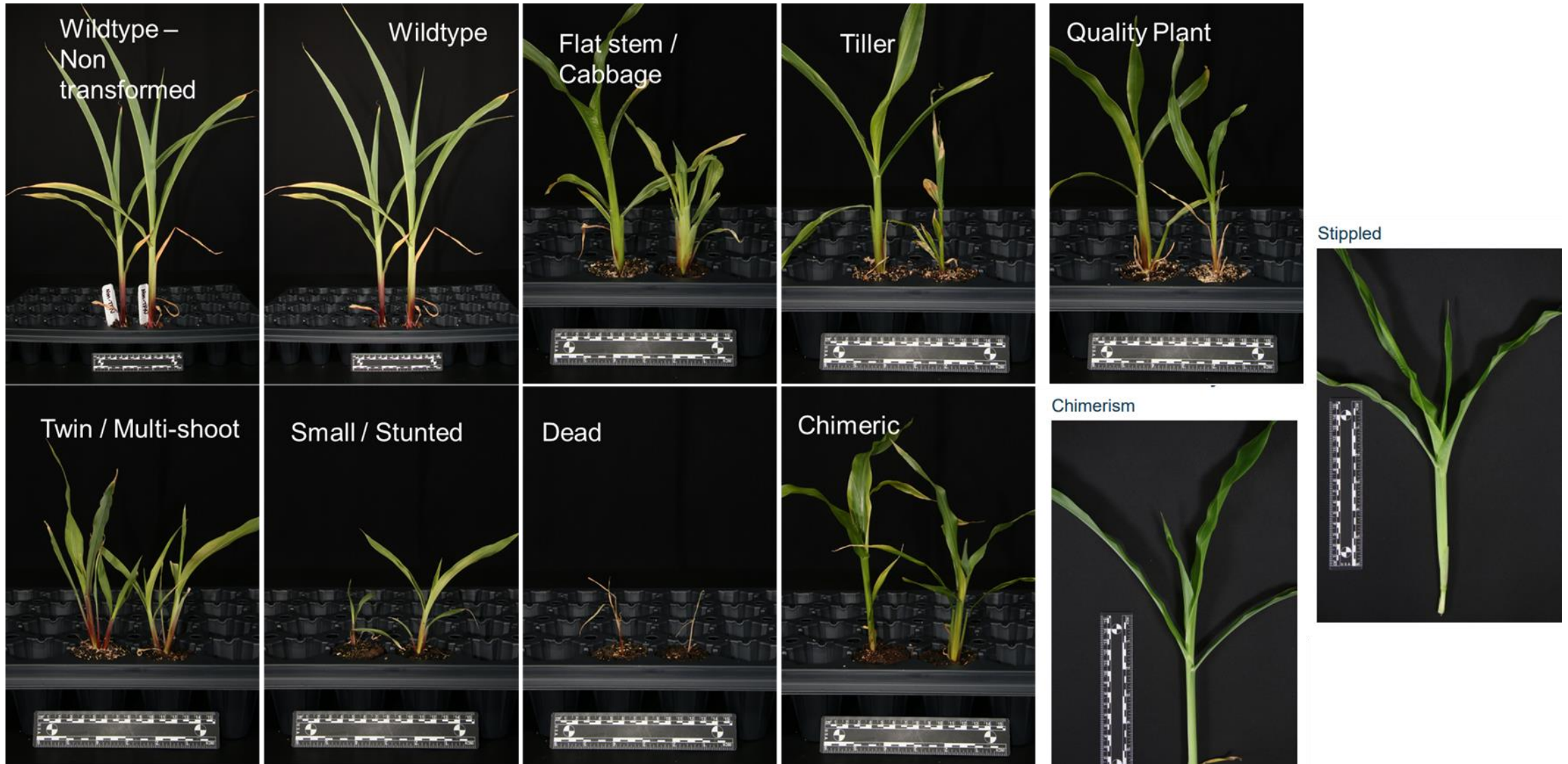
Suggested watering schedule with peat-based media

- Slow transition from tissue culture to greenhouse
- Day 1-5 mist and shade optional
- Sub-irrigation with 15-5-15 + Fe at 2,000 μ S
- Frequency varies by plant size and season

Time	Approx. developmental stage	Irrigation Type	Irrigation Time, Duration, & Frequency
Day 1-3	Plugging to V1	Sub-irrigation + Mist + Shade	0 x Daily + 15 sec. every 15 min + 30-40% shade
Day 3-5	V1	Sub-irrigation + Mist + Shade	1 x Daily + 15 sec. every 30 min + 30-40% shade
Day 6-8	V1-V2	Sub-irrigation	1-2 x Daily
Day 9-14	V2-V3	Sub-irrigation	2-3 x Daily
Day 15-21	V4 by Day 21. Transplant at end of week 3.	Sub-irrigation	Up to 4 x Daily



Corn R0 Plant Production: Pre-Transplant Phenotypes





Corn R0 Plant Production: Post-Transplant



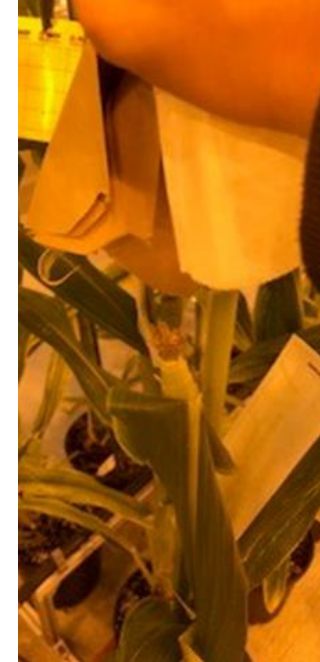
Early Production
- Rouging



Middle
Production -
Rouging



Tassel and Ear
Health



Pollination



Post Pollination -
Rouging

29.4/21.1 C (85/70 F) D/N
16 hour photoperiod
Fertilizer 15-5-15 at 2,000 μ S



Corn R0 Plant Production: Post-Transplant Phenotypes



Tiller



Malformed Leaves



Chimerism



Chimerism



Stippling



Poor Silk



Poor Pollen



Skeletonized Tassel



Feminized Tassel



No Ear/Did Not Nick



Smut



Mold



Corn R0 Plant Production: Post-Transplant

Suggested watering schedule with peat-based media before pollination is complete

- 400 ml per minute with spray emitters
- Wet/Dry cycles early to promote root development
- Visible ear shoot/tassels is stage for flush (week 6)
- Flush = hand watering with plain water 2 – 3 times to field capacity
- Avoid water stress after flush

Time	Approx. developmental stage	Approx. Irrigation Time	Days
week 3	Transplant at end of week 3. V4 by Day 21	Hand water	At transplant then as needed to V6
week 4	V5-V6 by day 28	0:20 sec (8 am)	Every other day (EOD); Every day (ED) by end of week 4
week 5	V6-V8 by day 35	0:20 sec (8 am) then 0:20 sec (8 am, 4pm)	Every day (ED)
week 6	V9-V11 by day 40. Some events may start to show a tassel by day 35; Other early-developing events may need self-pollination.	0:30 – 0:40 sec (8 am, 4 pm) plus end of week flush	Every day (ED)
week 7	VT-R1 by day 49. About 50% of events will require pollination	0:40 sec, 2-3 x, (8 am, 12 pm, 4 pm)	Every day (ED)
week 8	VT-R1 by day 56. Remainder of events will require pollination (40-50%). Week 6 pollinations = bag lift.	0:50 – 0:60 sec, (8 am, 12 pm, 4pm)	Every day (ED)
week 9	VT-R1. A few events may will require pollination (5-10%). Week 6 pollinations = peel back. Week 7 pollinations = bag lift	0:50 – 0:60 sec, (8 am, 12 pm, 4pm)	Every day (ED)



Corn R0 Plant Production: Post-Transplant

Suggested watering schedule with peat-based media after pollination

- 400 ml per minute with spray emitters
- R0 maturation will vary by pollination date.
- Irrigation management to pot level.
- Lower water frequency as seed matures to promote drying

Time	Approx. developmental stage	Approx. Irrigation Time	Days
week 9	VT-R1. A few events may will require pollination (5-10%). Week 6 pollinations = peel back. Week 7 pollinations = bag lift	0:50 – 0:60 sec, (8 am, 12 pm, 4pm)	Every day (ED)
week 10	Week 6 pollinations = reduced watering. Week 7 pollinations = peel back. Week 8 pollinations = bag lift.	0:50 – 0:60 sec, (8 am, 12 pm, 4pm)	Every day (ED)
week 11	Week 6 pollinations = no water. Week 7 pollinations = reduced watering. Week 8 pollinations = peel back. Week 9 pollinations = bag lift.	0:50 – 0:60 sec, (8 am, 12 pm, 4pm)	Every day (ED)
week 12	Week 6 pollinations = harvest. Week 7 pollinations = no water. Week 8 pollinations = reduced watering. Week 9 pollinations = peel back.	0:50 – 0:60 sec, (8 am, 12 pm, 4pm)	Every day (ED)
week 13	Week 7 pollinations = harvest. Week 8 pollinations = no water. Week 9 pollinations = reduced water.	0:50 – 0:60 sec, (8 am, 4pm)	Every day (ED)
week 14	Week 8 pollinations = harvest. Week 9 pollinations = no water	0:50 – 0:60 sec, (8 am) then none	Every day (ED)
week 15	Week 9 pollinations = harvest. All seed now drying or being processed.	none	Every day (ED)



Corn R0 Plant Production: Mature Seed



Late Production –
Seed Production

85/70 F (29.4/21.1 C) D/N
16 hour photoperiod
Fertilizer 15-5-15 at 2,000 μ S



Bag Lift



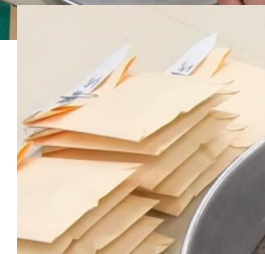
Peel Back



Seed
Maturation



Ear Harvest



Seed
Processing



R0 Production Summary: R0 Plants are different than Inbred Plants

- R0 Plants are less vigorous than Inbred plants of same genetic background
- R0 plant phenotypes are highly variable
- Early management decisions will impact large plant health
 - Water management
 - Light levels
 - Fertility levels
- Selection is essential for successful pollinations and quality seed
- Expect less than 40% of transformed plants to pass molecular, pass phenotypic rouging, and generate seed for next generation



Inbred

Quality R0



Inbred

R0



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Thank you!



Any questions?

