Increased Profits from Disease-Free Garlic Planting Stock
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Summary

The goal of this project was to increase garlic grower profits by teaching about the benefits of quality garlic seed. Over 300 garlic growers received information about workshops on Identification and Management of Garlic Pests and Diseases and over 650 garlic growers from Maine, New Hampshire, and New York attended 24 presentations on garlic. Five publications, a webinar/video and two YouTube videos were developed with over 49,000 page views during the time frame of this grant. Additionally, a website http://umaine.edu/agriculture/programs/garlic/ was developed with over 800 views during the time frame of this grant. As a result of this project, 80% of the garlic growers can now recognize major garlic diseases; 92% realize the importance of major garlic diseases in garlic production; and 71% of garlic growers changed their management strategy to look to plant nematode-free or disease-tested garlic seed stock.

Introduction

There are over 70 commercial garlic growers in Maine representing all 16 counties. There is no seed program present for disease testing. Importation of seed stock and exchange of live plant material has contributed to new pathogens being imported into and distributed within Maine. Two previously unreported pathogens in Maine on garlic have been imported on garlic seed stock. Losses on some affected farms exceeded 40 percent.

Garlic was put into tissue culture to produce garlic seed stock free from pathogens. While there was success producing tissue-culture generated garlic bulbs, scaling up to a commercial quantity proved to be difficult. Efforts are continuing beyond the grant to address laboratory hurdles in producing tissue-culture generated garlic bulbs. The seedlings had a difficult transition from the greenhouse to the field. Additionally, the seedlings needed longer to mature than the season allowed. The seedlings also did not produce bulbs with typical multiple cloves, but rather a single clove or a "round," indicative of a stressful situation. Efforts are continuing beyond the grant to address this situation as well.

Shortly after this project was started, a garlic bloat nematode epidemic appeared in New York and New England. As a result of the epidemic, the main garlic seed concern growers had was obtaining planting stock free from the garlic bloat nematode. Developing disease-free seed through tissue culture was the target of the project. Identifying garlic diseases as well as sources of disease-free garlic planting stock for growers as well as resellers became a priority for outreach efforts.

Objectives/performance targets

One hundred garlic farmers will recognize major garlic diseases and realize their importance in garlic production.

Materials and methods

Laboratory procedures:

There are great differences among garlic types regarding how well they adapt to tissue culture. Generally, the porcelains types tend to do better than the rocambole types. There is also a wide difference between isolated callus within a garlic

variety as well as isolated callus within a single clove slice. Learning the nuances of the different garlic cultivars is critical. Recognizing when callus tissue has exhausted the media and should be transferred onto fresh media is also critical.

Develop garlic callus cultures from a bulb:

Pull the cloves from the bulb and peel the protective wrapper off a clove and soak the clove in a 10% bleach solution for 5 minutes. With a sterilized scalpel, cut off and discard about 1 mm off the end of the basal plate as this is often a source of contamination. Using a sterilized scalpel, cut off slices 2 to 3 mm thick parallel to the basal end of the clove. Place these slices onto a plate of callus medium. In about 8 weeks in the dark at 20°C, callus growth appears at the edge of the slices. Generally, callus tissue can be expected to develop in 1 in 5 slices with fresh bulbs, with much less in older bulbs. Contamination is greatest closest to the basal plate and increases with age of bulbs. This is the stage with the greatest loss to contamination.

Callus medium (1 liter):

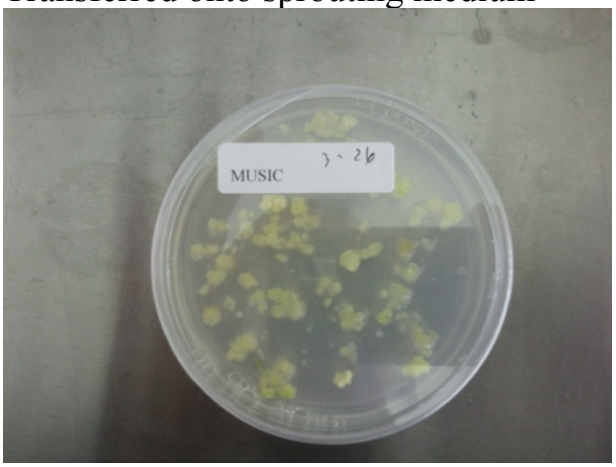
3.2 g	Gamborg's B-5 Basal Medium with Minimal Organics (Sigma G6593-10L)
30 g	Sucrose (Sigma S5801-5KG)
2.5 g	Phtyogel (Sigma P-8169-250g)
1 ml	6-(y, y-Dimethylallylamino) purine solution (2iP) (Sigma D7674-1G) {6-(y,y-Dimethylallylamino) purine solution (2iP) (50 ml): Dissolve 0.05 g in 5 ml NaOH; bring up to 50 ml}
1 ml	2,4-Diclorophenoxyacetic acid solution (2,4-D) (Sigma D7299-100G) {2,4-Diclorophenoxyacetic acid solution (2,4-D) (50 ml): Dissolve 0.05 g in 5 ml NaOH; bring up to 50 ml}

Adjust to pH 5.7 drop by drop with HCl

About 8 weeks of growth.

<u>Multiply garlic callus tissue cultures from newly developed cultures:</u>
When the callus growth extends out about 3 to 4 mm from the clove slice, remove the callus and streak them across a new plate filled with callus medium, leaving bits of callus cells distributed across the plate. In about 8 weeks in the dark at 20°C, these callus bits increase in size to about 1 cm in diameter. Generally, increase in callus tissue can be expected to be 1 to 5 times starting amount. Contamination loss does occur with this stage, but less so than the previous step.

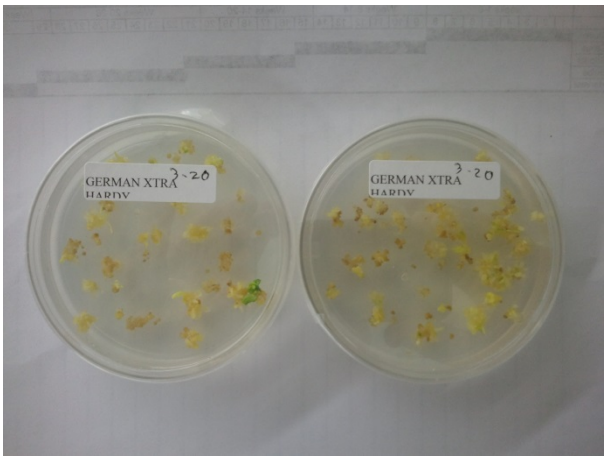
Transferred onto sprouting medium



Produce stems from garlic callus tissue cultures:

When the callus bits are about 1 cm in size, remove and streak them across a plate filled with stem medium, leaving bits of callus cells distributed across the plate. These plates require a 16-hour light, 8-hour dark cycle at 20°C. After about 8 weeks, sprouts begin to form from the callus tissue. Generally, stems can be expected to develop on about 60% of the transferred callus tissue. Contamination loss does occur with this stage, but less so than the previous step.

Transferred to stem medium



Stem medium (1 liter):

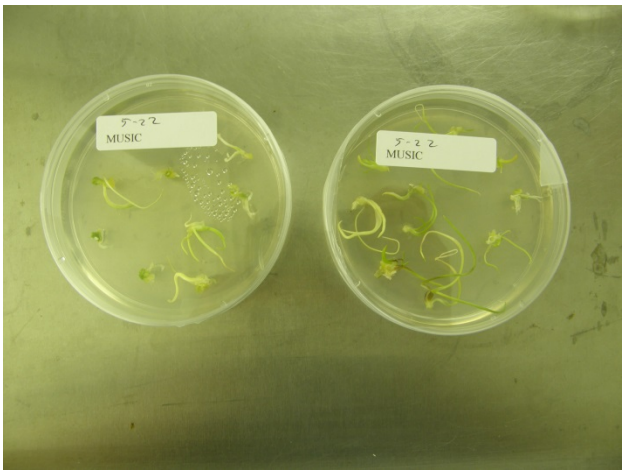
3.2 g	Gamborg's B-5 Basal Medium with Minimal Organics (Sigma G6593-10L)
30 g	Sucrose (Sigma S5801-5KG)
2.5 g	Phtyogel (Sigma P-8169-250g)
3 ml	6-Benzylaminopurine solution (BAP) (Sigma B3408-1G) {6-Benzylaminopurine solution (BAP) (50 ml): Dissolve 0.05 g in 5 ml NaOH; bring up to 50 ml}

Adjust to pH 5.7 drop by drop with HCl

Produce bulbs from garlic callus tissue cultures with stems:

Transfer intact callus tissue with developed sprouts to bulb medium. These plates require a 16-hour light, 8-hour dark cycle at 20°C. After about 4 weeks, bulbs will be initiated by the sprouted callus bits. Generally, bulbs can be expected to develop on about 60% of the transferred callus tissue with stems. Contamination loss does occur with this stage, but less so than the previous step. These bulbs are very small. After 8 weeks, the bulbs can be transferred to new plates filled with bulb medium which can increase their size.

Transferred to bulb medium



Bulb medium (1 liter):

4.4 g	Murashige and Skoog Basal Medium (Sigma M6899-1l) (MS)
120 g	Sucrose (Sigma S5801-5KG)

Adjust to pH 5.8 drop by drop with HCl

Greenhouse procedures:

Produce plants from garlic callus tissue bulbs:
Plant the tiny bulbs in a greenhouse in standard potting medium. Place cultures under standard grow lights providing 16-hour light, 8-hour dark period. Plants should be held at ambient temperature (20°C) and are slow to grow.

Field procedures:

Produce field-grown bulbs from greenhouse-grown garlic plants:
Transplant the greenhouse plants into the field when the danger of frost is past.

Results and discussion/milestones

Concern regarding the small size and overwintering ability of the bulbs was well founded. Just over 5 percent of the 200 planted bulbs survived. Plant growth was spindly and only 1 bulb differentiated into cloves. Most plants produced "rounds", indicative of stressful growing conditions.

Laboratory issues continued and scaling up to a commercial quantity proved to be difficult again. About 1000 tissue-culture generated garlic bulbs were produced. While this is more than the previous year, successfully growing the crop to bulbs had not been done so again, the decision was made to plant them at the University of Maine Experimental farm. The decision was made to increase tissue culture generated bulbs for spring planting in the greenhouse to increase the likelihood of survival.

Field issues continued to hinder the project. Less than 5% of the tissue culture generated bulbs survived and did not differentiate into a bulb with cloves. Limited survival of the garlic transplants from the green house occurred with not of the plant ready for harvest when harvest time arrived.

Impact of results/outcomes

Performance Target
One hundred garlic farmers will recognize major garlic diseases and realize their importance in garlic production and 30 farmers will adopt the new technique of planting pathogen-free garlic bulbs and their crop losses from introduced pathogens will be eliminated as will the spreading of these pathogens through their seed sales.

A garlic grower mailing list with 263 names on it was surveyed with a return rate of 25 percent, which generally is what is expected. Extrapolating the reported data to the population yields very encouraging results. 80% of the garlic growers can now recognize major garlic diseases; 92% realize the importance of major garlic diseases in garlic production; and 71% of garlic growers changed their management strategy to look to plant nematode-free or disease-tested garlic seed stock. This is well supported by garlic growers looking for clean planting stock. During the period of study there were 370 garlic bulb and soil samples submitted for pathogen testing as a result of the grant-related outreach efforts. While the samples were being tested for garlic bloat nematode, destructive diseases caused

by *Sclerotium cepivorum*, *Botrytis porri*, and *Fusarium* spp. were identified and information delivered to the grower on how to deal with the pathogen found on their sample. Many of these samples were a direct result of the educational outreach efforts. Numerous workshops on growing healthy garlic and recognizing diseases and what to do about them were well received. Pre- and post-workshop surveys revealed a 100% increase in garlic grower's ability to identify diseases. Crop rotation, disease identification, and planting bloat-tested stock were topics reported by attendees as practices that they will implement. Subsequent access of over 49,000 views of on-line educational efforts show a further interest in better understanding garlic diseases.

No farmers planted tissue-culture generated garlic bulbs owing to laboratory hurdles. Without significant breakthroughs in the laboratory, there is not a great deal of hope for tissue culture-generated garlic bulbs to be available on a commercial scale. Greenhouse-grown seedlings may be an alternative, but there are still questions as to the commercial viability of the process. Efforts are continuing to address these issues. Growers did however, plant garlic bulbs pathogen-free as currently available. This was accomplished through educational outreach activities and extensive laboratory testing. The 370 samples submitted for pathogen testing went a long way toward accomplishing disease-free garlic seed as some seed stocks were culled out as result of testing positive for some pathogens. As a result of the educational outreach efforts, growers were demanding certifications of health through laboratory testing of the seed stock they wanted to buy and plant and was made mandatory by major garlic seed resellers. We think the objective of one hundred garlic farmers recognizing major garlic diseases and realizing their importance in garlic production was soundly met. We also think the objective of 30 farmers adopting the new technique of planting pathogen-free garlic bulbs was soundly met.

Publications/outreach

As part of the project, 19 presentations on garlic were given to over 650 garlic growers from Maine, New Hampshire, and New York. Topics included garlic production, garlic diseases, and growing healthy garlic. In addition, five publications, a webinar/video and two YouTube videos were developed with over 49,000 page views during a recent time frame. Additionally, a website (http://umaine.edu/agriculture/programs/garlic/) was developed with over 840 views during the time frame of this grant. Owing to the UMaine webpage setup, views of the webinar/videos could not be tracked. These outreach efforts are detailed below.

Presentations:

Johnson, S. B. 2015. Garlic and Root Crops Storage: What Works and What Doesn't. Presented at the Empire State Producers Expo on January 20, 2015 in Syracuse, NY.

Fuller, D. 2014. Growing Healthy Garlic. Presented in Livermore Falls, ME on October 2, 2014.

Johnson, S. B. 2013. Growing Healthy Garlic. Presented at the Cape Elizabeth Garden Club Meeting in Cape Elizabeth, ME on October 24, 2013.

Johnson, S. B. 2013. Bloat Nematode in Maine Garlic -- A New Pathogen in Maine. Presented at the 73rd Meeting of the Northeast Division of the American Phytopathology Meeting in Southbury, CT on October 24, 2013.

Fuller, D. 2013. Growing Healthy Garlic and Dealing with Old and New Diseases. Presented in Lowell, ME on October 17, 2013.

Fuller, D. 2013. Growing Healthy Garlic and Dealing with Old and New Diseases. Presented in Rangeley, ME on October 1, 2013.

Fuller, D. 2013. Growing Healthy Garlic and Dealing with Old and New Diseases. Presented at the Farmington Seed Savers 3rd Annual Winter Conference on February 2, 2013 in Farmington, ME.

Johnson, S. B. 2013. Growing Healthy Garlic: Disease and Storage Issues. Presented in Deer Isle, ME on December 4, 2013.

Fuller, D. 2013. Growing Healthy Garlic. Presented in Deer Isle, ME on December 4, 2013.

Fuller, D. 2013. Maine Garlic Research Update. Presented at the Maine Chapter of the National Association of Agricultural Agents in Orono, ME on November 25, 2013.

Fuller, D.F. 2012. Testing of Seed Garlic for Common Ground Fair Seed Garlic Sellers. Presented at the Farmer's Market Steering Committee Meeting on December 13, 2012 in Unity, ME.

Johnson, S. B. 2012. Pest Biology and Identification. Presented at the Farmer's Market Steering Committee Meeting on December 13, 2012 in Unity, ME.

Johnson, S. B**.** 2012. Garlic: An update on Production and Dealing with Old and New Pests. Presented at the Maine Agricultural Trades Show on January 10, 2012 in Augusta, ME.

Johnson, S. B. 2012. Disease issues for Garlic and other Bulb Crops. Presented at the Maine Agricultural Trades Show on January 11, 2012 in Augusta, ME.

Johnson, S. B. 2012. Disease Issues for Garlic. Presented at the First Annual Capital District Garlic School on March 26, 2012 in Saratoga Springs, NY.

Johnson, S. B. 2012. Disease Issues for Garlic. Presented at the First Annual Geneva Garlic School on March 27, 2012 in Geneva, NY.

Johnson, S. B. 2012. Disease Issues for Garlic. Presented at the First Annual Ithaca Garlic School on March 28, 2012 in Ithaca, NY.

Fuller, D.F. 2012. Garlic Production Update and Dealing with Old and New Pests. Presented at the Common Ground Country Fair, September 21, 2012 in Unity, ME.

Johnson, S. B. 2012. Garlic Diseases. Presented at the North Country Fruit and Vegetable Seminar and Trade Show on October 30, 2012 in Whitefield, NH.

Publications:

Johnson, S. B. and Fuller, D. 2014. Bloat Nematode in Maine Garlic – A New Pathogen in Maine. Phytopathology 104:3.

Fuller, D. F. and Johnson, S. B. 2013. The Maine Garlic Project: A Participatory Research and Education Program. Journal of Extension [On-line], 50(5) Article 5TOT8. Available at: http://www.joe.org/joe/2013august/tt6.php

Johnson, S. B. and Fuller, D. 2013. Blue Mold of Garlic. University of Maine Cooperative Extension Bulletin, 1206, 2pp. Available at: http://umaine.edu/publications/1206e/

Fuller, D and Johnson, S. B. 2013. Growing Hardneck Garlic in Your Maine Garden. University of Maine Cooperative Extension Bulletin, 2063, 3pp. Available at: http://umaine.edu/publications/2063e/

Johnson, S. B. and Fuller, D. 2013. Botrytis Neck Rot in Maine Garlic, 1207, 2pp. Available at: http://extension.umaine.edu/publications/1207e/

Webinars/Videos:

Johnson, S. B. and Fuller, D. 2013.Growing Healthy Garlic: Curing and Disease Issues. (http://www.umext.maine.edu/potatoprogram/garlic/Growing%20Healthy%20Garlic%20Curing%20and%20Disease%20Issues.wmv)

Web Page:

http://umaine.edu/agriculture/programs/garlic/

Farmer adoption

The two largest garlic seed resellers in Maine? are now requiring their growers to have their garlic tested for diseases before they are willing to market it. Independent garlic seed buyers were asking sellers for proof that their garlic had been tested before buying. Many growers reported that they could not sell their garlic for seed had they not attended workshops, learned about pathogen testing, and had their crop tested. There was no garlic seed testing program in Maine before this project began.

A sampling of feedback quotes from farmers received:

"I'll put this information to use immediately"

"A number of my garlic contemporaries in other states are jealous of the service and expertise that I have access to via Fuller and Johnson."

"After attending your talk, I sold $7,000 worth of garlic seed in 3 days as a result of having my garlic seed tested for diseases. Thanks Dave, for the great presentation."

“That same attention to the best science regarding propagation, diseases and pests, and “seed” quality can truly build a Garlic “seed” grower base that the rest of the country desperately needs.”

“The research Fuller and Johnson conduct is key to education, product quality, and market development.”

“Many buyers from other states recognize disease and pest analysis by Johnson as a gold standard of product quality assurance. A number of farm and garden mail-order businesses currently rely on the testing, research, and council of Fuller and Johnson to guide and grow garlic sales.”

Areas needing additional study

Any progress overcoming laboratory hurdles in tissue culture prosecution may make the practice viable for the future. Further investigation on the value of disease-free garlic seed to growers should continue.