

Delayed Senescence and Disease Tolerance of Tomato Plants Cultivated in Cover Crop Mulch Correlates with Accumulation of Specific Gene Products

Vinod Kumar¹, Douglas J. Mills², James D. Anderson³ and Autar K. Mattoo^{1,*}

¹ United States Department of Agriculture, ARS's H.A.W. Beltsville Agricultural Research Center-W, Building 010A, Vegetable Laboratory, Beltsville, MD 20705-2350, USA

(*Corresponding author)

² Weed Science Laboratory and ³ Plant Sciences Institute, Beltsville, MD 20705-2350 USA

Keywords: Mulch, vetch, plastic, *Lycopersicon esculentum*, defoliation, disease, proteins

Abstract

Vegetable production is heavily dependent upon high off-farm inputs of polyethylene (plastic) mulch, nitrogen fertilizer and pesticides. Such a production practice potentially contributes to the unintentional environmental pollution with fertilizer and pesticides as well as water run off and soil erosion. This raises serious environmental concerns for human and animal health. The integration of on-farm biological inputs into vegetable production system is one potential means of reducing the dependence on off-farm inputs. In recent years, alternative agriculture practices have tested cover crops like hairy vetch (*Vicia villosa*) as on-farm biological inputs that have the potential to reduce both erosion and the use of agrochemicals without impacting the yield or quality of the produce. Field-grown, fresh market tomato (*Lycopersicon esculentum* L.) plants cultivated in hairy vetch mulch display reduced defoliation and tolerance to disease as compared to plants cultivated in the plastic mulch. We have initiated a molecular approach to test whether these beneficial attributes are linked to changes in the expression profiles of one or more specific gene products. The data indicated that vetch-grown tomato plants have increased accumulation of transcripts and proteins that are central to delayed senescence and disease suppression.

INTRODUCTION

US consumer demand for fresh market vegetables has increased considerably in recent years due to their contribution to human health. Vegetables and fruits are rich sources of vitamins, minerals and fiber. Conventional production methods have met these demands but compromised on the environmental and social issues because these production systems heavily rely on materials synthesized off the farm, such as polyethylene mulch, nitrogen fertilizer and pesticides. The current reliance on off-farm inputs will continue unless profitable, lower-input systems for fresh market production are developed. The integration of on-farm biological inputs into vegetable production systems is one potential means of reducing the current dependence on off-farm inputs. Cover crops represent on-farm biological inputs that have the potential to reduce the use of polyethylene mulch, fertilizer and pesticides (Abdul-Baki et al., 1996a, b; Creamer et al., 1996). The benefits of cover crops include improvements in soil preservation (Flach, 1990), soil health (McVay et al., 1989), weed suppression (Teasdale, 1993; Creamer et al., 1996), and the reduction of soilborne diseases (Rothrock et al., 1995; Candole and Rothrock, 1997).

Hairy vetch has been used successfully as cover crop/organic mulch for growing processing tomato (Abdul-Baki et al., 1996a). In most cases, marketable yield of fresh market tomatoes was equivalent or greater in hairy vetch mulch as compared to black polyethylene mulch (Abdul-Baki et al., 1996a; Mills et al., 2002b). In addition, vetch decomposition provided nitrogen reducing the need for commercial nitrogen input (Abdul-Baki et al., 1997); nitrogen content of hairy vetch aerial biomass has been estimated at 126-169 kg ha⁻¹. Fresh market tomato production in hairy vetch mulch is

economical as compared to production using black polyethylene mulch (Kelly et al., 1995).

Cover cropping has been shown to suppress disease in some vegetable and fruit crops. The incidence of *Phytophthora* blight was reduced in peppers grown in a wheat cover crop compared to bare soil and polyethylene mulch (Ristaino et al., 1997). This reduction in disease incidence affected dispersal of propagules of *Phytophthora capsici*, the causal agent of *Phytophthora* blight (Ristaino et al., 1997). Sudangrass was shown to reduce the splash dispersal of *Colletotrichum acuatum* conidia and has the potential to manage anthracnose of strawberry (Ntahimpera et al., 1998). Compared to no soil cover, sand and plastic mulch, the incidence of leather rot disease of strawberry was reduced by using straw as a soil cover to reduce the splash dispersal of *P. cactorum* (Madden and Ellis, 1990). Cotton grown in hairy vetch (*Vicia villosa* Roth)-amended soil exhibited reduced incidence of black root rot, caused by *Thielaviopsis basicola* (Candole and Rothrock, 1997). The reduction of black root rot incidence correlated with increased ammonia levels in the soil (Candole and Rothrock, 1997). Compared to winter fallow, reduced soil levels of *T. basicola* were observed in a hairy vetch cover crop (Rothrock et al., 1995). Similarly, foliar disease was reduced in tomato plants grown in hairy vetch compared to bare soil (Mills, et al., 2002a). In addition, fruit produced in hairy vetch mulch appeared to be more suitable for fresh-cut slices than those grown in black plastic (Hong, et al., 2000).

The advantages of cover crop cultivation seem impressive. However, not much is known about the cellular processes impacted, signaling factors involved or molecular mechanisms that are fundamental to vetch-induced longevity and disease-suppression. To help understand these mechanisms, we initiated studies to identify genetic components that are regulated in plants cultivated in vetch-mulch. This report presents our findings to date.

HYPOTHESIS

Our hypothesis is that controlled release of carbon and nitrogen metabolites during water-mediated decomposition of vetch results in a certain carbon/nitrogen (C/N) ratio that influences expression of genes associated with senescence, longevity and/or disease infestation. This hypothesis is based on the following observations in the literature:

1. Sugars impact photosynthesis, nitrogen metabolism, sucrose and starch metabolism, and defense mechanisms by regulating a few key genes (Sheen, 1994; Koch, 1996; Sheen and Jang, 1997).
2. Nitrate regulates carbon metabolism, resource allocation, root development and flowering (Koch, 1997; Wang et al., 2000).
3. Nitrate down-regulates genes involved in starch synthesis, facilitating the mobilization of carbon skeletons into organic acids (Koch, 1997, Wang et al, 2000).
4. Nitrate is readily reduced to ammonia and subsequently incorporated into amino acids (Koch, 1997; Stitt, 1999).
5. Carbon : nitrogen (C/N) ratio may regulate metabolic development (Smeekens, 2000; Hellmann et al., 2000; Vidmar et al., 2000., Coruzzi and Zhou, 2001).

RESULTS AND DISCUSSION

Severer Foliar Disease Levels and Leaf Defoliation Occur in Black Polyethylene than in Hairy Vetch Mulch

The onset of foliar disease was significantly delayed in hairy vetch-grown plants compared to those cultivated on black polyethylene. For example, foliar disease in plastic beds exceeded 41% at 84 days after transplant compared to hairy vetch beds that showed remarkably little sign of disease onset during a field test in year 2000. Similarly, plants grown in black polyethylene beds lost ~33% of their foliage at 84 days after transplant whereas those grown in hairy vetch beds lost only ~11% of their foliage in the same time frame (Fig. 1).

Delay in Leaf Senescence in Hairy Vetch Tomato Plants Correlates with Delayed Disappearance of Senescence-Related Proteins

We used protein fractionation on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in conjunction with immunoblots as a first step towards cataloging differential changes, if any, in the steady-state levels of proteins isolated from plastic or vetch grown tomato leaves. Soluble and membrane proteins isolated from leaf tissues of plants at different developmental stages were fractionated on linear gradient (10-20%) acrylamide gels (Mattoo et al., 1981). The fractionated proteins were either stained with Coomassie Brilliant Blue or electrotransferred to insoluble matrices for immunoblot analysis.

To ascertain that delayed senescence in the vetch-grown plants is reflected by changes in the known, senescence-marker proteins, we employed antibodies raised against ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco), cytosolic glutamine synthetase (GS-1), and carboxypeptidase. No marked differences were apparent when the protein gels were stained with Coomassie blue. However, immunoblots revealed interesting data.

The level of nuclear-encoded small subunit of Rubisco declined by nearly 70% in plants grown under polyethylene compared to 32% in vetch-grown plants by 108 days after transplant. Under these conditions, the level of plastid-encoded large subunit of Rubisco decreased by about 78% in plants grown under polyethylene compared to ~5% in vetch-grown plants. Likewise, levels of GS-1 appeared to remain stable for a longer period in vetch-grown plants compared to those grown under polyethylene.

In comparison, the pattern of vacuole localized carboxypeptidase, implicated in the degradation of reserve proteins (Mehta et al., 1996), was similar in vetch-grown and polyethylene-grown plants. Similar patterns with no differential accumulation between the two cultivation conditions were found for such proteins as α -ATPase, β -ATPase, CP43, plastocyanin, cyt b_{559} , and PR-P, Q.

These data confirm that indeed the two important proteins, Rubisco and GS-1, whose disappearance is an early indicator of senescence (Mehta et al., 1992), are long-lived in vetch-grown plants compared to the conventionally cultivated plants. Further, results with other antibodies indicated that several other proteins are not modulated in this way. The questions are: what is the signaling pathway and what components in the hairy vetch soil regulate the expression of the beneficial genes? Do roots interact with soil microflora to generate a signal that is translocated to the foliage? Alternatively, are the beneficial responses exerted merely by differences in C/N levels?

CONCLUSION

We confirm here that tomato plants grown in hairy vetch mulch display increased crop longevity perhaps due to delayed senescence. In addition, we have observed that they have reduced foliar disease. These observations have important yield implications and are consistent with earlier studies (Abdul-Baki, et al., 1996a).

Glutamine synthetase (GS-1) catalyzes the first major step converting nitrogen into an organic form (Lam et al., 1995). Its stable nature in vetch-grown plants is reflected in delayed senescence. Therefore, it is a good candidate for rate limiting nitrogen assimilation and subsequent incorporation into proteins. Over-accumulation accompanied with higher stability of a few gene products central to senescence suggests that a select group of genes responds to changes in environmental signals associated with hairy vetch mulch. Upon degradation under moist conditions, hairy vetch mulch alters the carbon and nitrogen balance in soils. Yeast and bacteria have developed mechanisms to respond to changes in the levels of carbon and nitrogen metabolites, commonly referred as C:N-sensing. Such carbon- and nitrogen-mediated sensing mechanisms regulate the expression of a wide range of genes known to participate in growth and development (Coruzzi and Zhou, 2001). Why this regulation is restricted only to a few genes remains a focal point of our future investigation. Our current efforts on PCR-select cDNA subtraction and DNA microarray analysis of transcripts derived from vetch and plastic grown tomato can provide a useful tool in our attempts to isolate regulatory group of genes (Fig. 2).

Literature Cited

- Abdul-Baki, A.A., Stommel, J.R., Watada, A.E., Teasdale, J.R. and Morse, R.D. 1996a. Hairy vetch mulch favorably impacts yield of processing tomatoes. *HortSci.* 31:338-340.
- Abdul-Baki, A.A., Teasdale, J.R., Korcak, R., Chitwood, D.J. and Huettel, R.N. 1996b. Fresh-market tomato production in a low-input alternative system using cover-crop mulch. *HortSci.* 31:65-69.
- Abdul-Baki, A.A., Teasdale, J.R. and R.F. Korcak. 1997. Nitrogen requirements of fresh-market tomatoes on hairy vetch and black polyethylene mulch. *HortSci.* 32:217-221.
- Candole, B.L. and Rothrock, C.S. 1997. Characterization of the suppressiveness of hairy vetch-amended soils to *Thielaviopsis basicola*. *Phytopath.* 87:197-202.
- Coruzzi, G.M. and Zhou, L. 2001. Carbon and nitrogen sensing and signaling in plants: emerging 'matrix effects'. *Curr. Opin. Plant Biol.* 4:247-253.
- Creamer, N.G., Bennett, M.A., Stinner, B.R. and Carding, J. 1996. A comparison of four processing tomato production systems differing in cover crop and chemical inputs. *J. Amer. Soc. Hort. Sci.* 121:559-568.
- Flach, K.W. 1990. Low-input agriculture and soil conservation. *J. Soil Water Conserv.* 45:42-47.
- Hellmann H, Funck D, Rentsch, D and Frommer, W.B. 2000. Hypersensitivity of an *Arabidopsis* sugar signaling mutant toward exogenous proline application. *Plant Physiol.* 123:779-790.
- Hong, J.H., Mills, D.J., Coffman, C.B., Anderson, J.D., Camp, M.J. and Gross, K.C. 2000. Tomato cultivation systems affect subsequent quality of fresh-cut fruit slices. *J. Amer. Soc. Hort. Sci.* 125: 729-735.
- Kelly, T.C., Lu, Y., Abdul-Baki, A.A. and Teasdale, J.R. 1995. Economics of a hairy vetch mulch system for producing fresh-market tomatoes in the mid-Atlantic region. *J. Amer. Soc. Hort. Sci.* 120:854-860.
- Koch, K.E. 1996. Carbohydrate-modulated gene expression in plants, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47:509-540.
- Koch, K.E. 1997. Molecular crosstalk and the regulation of C- and N-responsive genes. In: *A Molecular Approach to Primary Metabolism in Higher Plants*, C.H. Foyer and W.P. Quick, eds (London: Taylor and Francis), pp. 105-124.
- Lam, H.-M., Coschigano, K., Schultz, C., Melo-Oliveira, R., Tjaden, G., Oliveira, I., Ngai, N., Hsieh, M.-H. and Coruzzi, G. 1995. Use of *Arabidopsis* mutants and genes to study amide amino acid biosynthesis. *Plant Cell* 7:887-898.
- Madden, L.V. and Ellis, M.A. 1990. Effect of ground cover on splash dispersal of *Phytophthora cactorum* from strawberry fruits. *J. Phytopath.* 129:170-174.
- Mattoo, A.K., Pick, U., Hoffman-Falk, H. and Edelman, M. 1981. The rapidly metabolized 32,000-dalton polypeptide of the chloroplast is the 'proteinaceous shield' regulating photosystem II electron transport and mediating diuron herbicide sensitivity. *Proc. Natl. Acad. Sci. U.S.A.* 78: 1572-1576.
- Mehta, R.A., Fawcett, T.W., Porath, D. and Mattoo, A.K. 1992. Oxidative stress causes rapid membrane translocation and *in vivo* degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase. *J. Biol. Chem.* 267:2810-2816.
- Mehta, R.A., Warmbardt, R.D. and Mattoo, A.K. 1996. Tomato (*Lycopersicon esculentum* cv. pik-red) leaf carboxypeptidase: identification, N-terminal sequence, stress-regulation, and specific localization in the paraveinal mesophyll vacuoles. *Plant Cell Physiol.* 37:806-815.
- McVay, K.A., Radcliffe, D.E. and Hargrove, W.L. 1989. Winter legume effects on soil properties and nitrogen fertilizer requirements. *Soil Sci. Soc. Amer. J.* 53:1856-1862.
- Mills, D.J., Coffman, C.B., Teasdale, J.R., Everts, K.L. and Anderson, J.D. 2002a. Factors associated with foliar disease of staked fresh market tomatoes grown under differing bed strategies. *Plant Dis.* 86:356-361.
- Mills, D.J., Coffman, C.B., Teasdale, J.R., Everts, K.L., Abdul-Baki, A.A., Lydon, J. and Anderson, J.D. 2002b. Foliar disease in fresh-market tomato grown in differing bed

- strategies and fungicide spray programs. *Plant Dis.* 86:955-959.
- Ntahimpera, N., Ellis, M.A., Wilson, L.L. and Madden, L.V. 1998. Effects of a cover crop on splash dispersal of *Colletotrichum acutatum* conidia. *Phytopath.* 88:536-543.
- Ristaino, J.B., Parra, G., and Campbell, C.L. 1997. Suppression of *Phytophthora* blight in bell pepper by a no-till wheat cover crop. *Phytopath.* 87:242-249.
- Rothrock, C.S., Kirkpatrick, T.L., Frans, R.E. and Scott, H.D. 1995. The influence of winter legume cover crops on soilborne plant pathogens and cotton seedling diseases. *Plant Dis.* 79:167-171.
- Smeekens, S. 2000. Sugar-induced signal transduction in plants. *Plant Mol. Biol.* 51:49-81.
- Stitt, M. 1999. Nitrate regulation of metabolism and growth. *Curr. Opin. Plant Biol.* 2:178-186
- Sheen, J. 1994. Feedback control of gene expression, *Photosynth. Res.* 39:427-438.
- Sheen, J. and Jang, J-C. 1997. Sugar sensing in higher plants. *Trends Plant Sci.* 2:208-214.
- Teasdale, J.R. 1993. Interaction of light, soil moistures, and temperature with weed suppression by hairy vetch residue. *Weed Sci.* 41:46-51.
- Vidmar, J.J., Zhuo, D., Siddiqi, M.Y., Schjoerring, J.K., Touraine, B. and Glass, A.D. 2000. Regulation of high-affinity nitrate transporter genes and high-affinity nitrate influx by nitrogen pools in roots of barley. *Plant Physiol.* 123:307-318.
- Wang, R., Guegler, K., LaBrie, S.T. and Crawford, N.M. 2000. Genomic analysis of a nutrient response in *Arabidopsis* reveals diverse expression patterns and novel metabolic and potential regulatory genes induced by nitrate. *Plant Cell* 12:1491-1509.

Figures

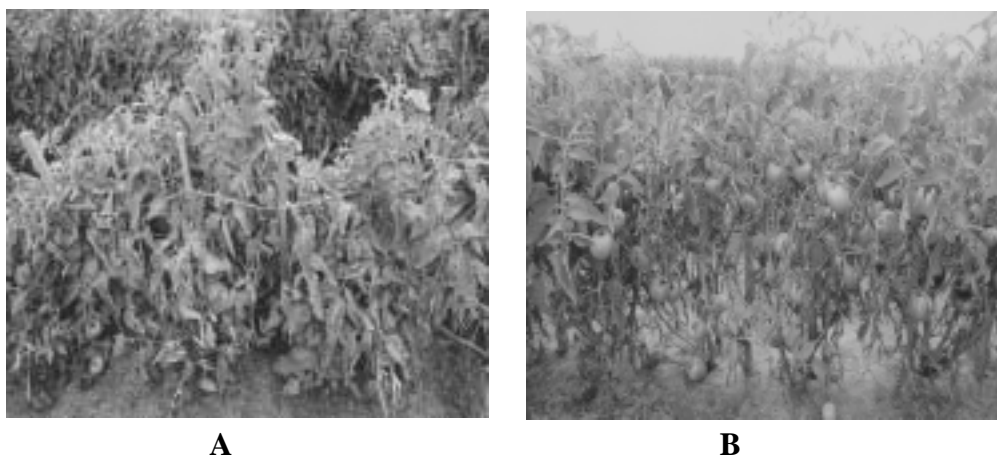


Fig. 1. Field grown tomato plants 82-days after transplant on plastic (A) and vetch (B) mulch.

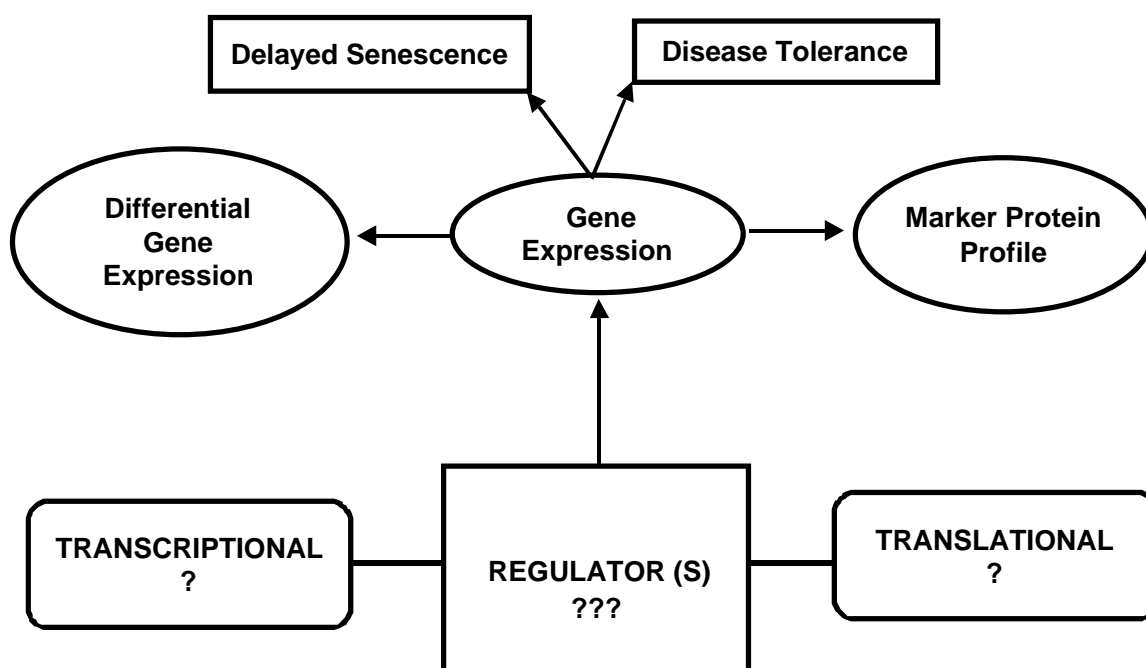


Fig. 2. A model representing the molecular road map in identifying critical genes involved in disease tolerance and delayed defoliation of vegetables produced through sustainable farming practices. This model is based on the assumption that vetch mulch-induced beneficial attributes in tomato plants are controlled at transcriptional and/or translational levels by a group of regulators, resulting in higher steady-state levels of key proteins that are central to disease suppression, increased longevity and delayed senescence.