Experiment V was designed with the objective of identifying minimum seed required to obtain maximum biomass yield. Hence, different seed rates at 300, 400 and 500 g/ft^2 were tested to produce hydroponic fodder maize; Table 6 presents the results.

The biomass yield recorded after nine days was found to be significantly higher in 300 and 400 g/ft^2 compared to 500 g/ft^2. This study reveals that increasing the seed rate affects the biomass yield. Thus, 300 g/ft^2 was found to be the optimum seed rate for hydroponic fodder maize cultivation. Naik *et al.*[8] have reported that high seed density increases the chances of microbial contamination in the root mat, which in turn affects the growth of the sprouts.

Experiment VI was conducted to compare the machine and manual shelled maize seeds for biomass yield of hydroponic fodder maize; Table 7 provides the results.

The biomass yield recorded after nine days was found to be higher in manually harvested maize seeds (9%). This may be because of poor germination due to damaged seeds during machine shelling.

The optimized conditions for the growth of hydroponic fodder maize are: (i) base materials are not required; (ii) 70% shade net as roof material; (iii) 2.5% biogas slurry as a nutrient; (iv) no additional night light required; (v) seed rate of 300 g/ft^2 and (vi) usage of manual shelled maize seeds. Hence by adopting these optimized conditions, hydroponic fodder maize can be produced during fodder scarcity for sustainable livestock farming.

1. GoI, Vision 2030. 11th Five-Year Plan document, Government of India, 2009.
2. Butler, J. D. and Oebker, N. F., Circular 844. Information Office, College of Agriculture, University of Illinois, Urbana, USA, 1962.
3. Butler, J. D. and Oebker, N. F., Circular 844, Information Office, College of Agriculture, University of Illinois, Urbana, USA, 2006.
4. Kide, W., *J. Agric. Vet. Sci*., 2015, **8**(7), 24–30.
5. Nugroho, H. D., Permana, I. G. and Despal, D., *Media Peternakan*, 2015, **38**(1), 70–76.
6. AOAC, *Official Method of Analysis*, Association of Official Analytical Chemist, Arlington, VA, USA, 2012, 21st edn.
7. Morgan, J., Hunter, R. R. and Haire, R. O., In 8th International Congress on Soilless Culture, Hunter's Rest, South Africa, 1992.
8. Naik, P. K., Swain, B. K., Swain, N. P. and Singh, N. P., *Indian J. Anim. Nutr*., 2015, **32**(1), 1–9.

ACKNOWLEDGEMENT. We thank the Tamil Nadu Veterinary and Animal Sciences University, Chennai for support under the TANUVAS Corpus Research Fund to carry out this work.

Received 9 September 2018; accepted 6 December 2018

S. GUNASEKARAN[1,*]
C. BANDESWARAN[2]
C. VALLI[1]

[1]*Institute of Animal Nutrition, Kattupakkam, and*
[2]*Department of Animal Nutrition, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Chennai 600 051, India*
**For correspondence.*
e-mail: gunaj2@gmail.com

Enhancing the efficiency of detached leaf method for resistance breeding in apple by considering leaf emergence phenology

Apple (*Malus* × *domestica* Borkh.) is the most important fruit crop of temperate regions. Jammu and Kashmir (J&K), Uttarakhand, Himachal Pradesh, Arunachal Pradesh and Nagaland are apple-producing states in India. Apple industry provides livelihood to millions of people of these states. J&K contributes about 60–65% of the total production in the country, providing livelihood security to a large section of the people of the state[1]. However, people associated with the apple industry face heavy losses (up to 70% reduction in crop production in severe cases) every year as the crop is prone to various diseases resulting in huge economic losses[2]. Alternaria leaf blotch caused by *Alternaria alternata* and apple scab caused by the fungal pathogen *Venturia inaequalis* are the two important diseases of the fruit crop. Alternaria leaf blotch of apple results in irregular light-brown spots on the leaves bordered by dark brown to purple margins[3]. The *V. inaequalis* infection results in undesirable changes in the shape and size of the fruits, affecting both appearance and quality of the infected fruits. It also leads to premature fruit fall and defoliation, besides making the tree sensitive to freezing and chilling injuries[4]. For managing these two diseases, several fungicide sprays are applied every year, which is not only expensive but also ecologically hazardous. Furthermore, residues get accumulated in the fruits, which have ill-effects on the consumers' health[5]. Consumers prefer fresh, blemish-free fruits devoid of any harmful chemicals. Resistance breeding is the best strategy to manage these diseases. The development of high-quality disease-resistant varieties is a major objective of apple breeding projects worldwide. European project HIDRAS (www.hidras.com) is a prime example. Once a disease-resistant variety is identified, it can be directly distributed to farmers provided it has fruit quality attributes that meet the demands of consumers. On the other hand, if the variety fails in terms of fruit quality attributes, it can be used in breeding programmes to transfer resistance in an otherwise susceptible variety having better fruit qualities through various breeding methods. However, the first step is the identification of disease-resistant varieties (sources). This calls for screening of the germplasm. Such screening is carried out at both phenotypic as well as genotypic levels. For genotypic screening, markers closely linked to disease resistance genes are used[6]. Phenotypic screening is carried

out by artificial inoculation of plantlets with spore suspension of the pathogen in glasshouse conditions followed by symptom assessment[7]. However, the method is expensive and time-consuming. Another method for screening at phenotypic level is the detached leaf method, wherein detached leaves are inoculated with fungal suspension followed by symptom assessment. Currently, the detached leaf method developed by Abe *et al*.[8] is widely used. Here, we suggest as to how the method can yield more reliable results by taking leaf emergence phenology into consideration.

Abe *et al*.[8] proposed a method for screening varieties of apple and other *Malus* species to identify resistance sources to Alternaria leaf blotch. It is also used in screening resistance sources to apple scab. The primary difference is the type of spore suspension used for inoculation. Once the spore suspension of the pathogen is made, it is used to inoculate the detached leaves of varieties used in the screening. The leaves are then kept in petri plates, and a wet paper is put inside the plate to provide high humidity. The plates are then transferred to incubators, and after 48 h the leaves are assessed for symptoms. Abe *et al*.[8] used detached leaves at different positions from growing shoots and inoculated them with the spore suspension of *A. alternata*, and upon symptom assessment, they concluded that the second leaf from the top of growing shoots gave the best results regarding screening for resistance sources to Alternaria leaf blotch. The inoculum was prepared from infected leaves of a susceptible cultivar and multiplied on potato dextrose agar (PDA) medium for 7–10 days at 25°C under continuous fluorescent light. Out of the five top leaves from the growing shoots of different apple genotypes, they found that the second leaf from the top gave the best results upon inoculation with the conidial suspension. The second youngest leaf from the top showed consistent results compared to other leaves, leading them to recommend that the second youngest leaf should be inoculated with spore suspension in order to identify resistance sources to Alternaria leaf blotch of apple.

Since apple is a temperate fruit tree, there is every chance that different varieties may show leaf emergence at different times in a particular region, as is the case with most temperate tree species[9]. Besides, apple cultivars have different chilling requirements to fulfil endodormancy, which is a prerequisite for leaf and flower bud emergence, indicating that there must be a clear difference in the timing of leaf emergence of these varieties[10]. Thus even if the second youngest leaves of different apple cultivars are used (as proposed by Abe *et al*.[8]), at the time of inoculation the leaves would be of different ages. Age difference between leaves implies differences in the ontogenic resistances. This difference may thus give incorrect results upon inoculation with the spore suspension. Furthermore, all the leaves of a tree do not emerge simultaneously, it takes a few weeks for all of them to emerge[11]. Thus second leaves of two shoots of the same plant may have different ages and different levels of ontogenic resistance. We suggest that leaf emergence phenology should be carried out, and genotypes should be grouped on the basis of simultaneous leaf emergence. To record leaf emergence dates, field visits need to be performed in early spring. After group formation, we should select those shoots from members of a group that show simultaneous leaf emergence. Preferably we can use shoots on which first leaf emergence takes place because it will then not be necessary to monitor other shoots. We can select second leaves from the top of those particular shoots for inoculation tests. This would remove the effect of leaf age and ontogenic resistance, and the results would be more reliable. The method may be useful for screening other temperate plants like peach, apricot, almond, etc. to identify resistance sources to various fungal diseases.

1. Anon., India Horticulture Database Millennium, National Horticulture Board, Ministry of Agriculture, Government of India, 2000.
2. Jha, G., Karnika, T. and Priyanka, T., *J. Biomed. Biotech.*, 2010, **10**, article ID 680160.
3. Filajdić, N. and Sutton, T. B., *Plant Dis.*, 1991, **75**(10), 1045–1048.
4. Gessler, C., Patocchi, A., Sansavini, S., Tartarini, S. and Gianfranceschi, L., *Crit. Rev. Plant Sci.*, 2006, **25**(6), 473–503.
5. Lozowicka, B., *Total Environ.*, 2015, **502**, 184–198.
6. Patocchi, A., Frei, A., Frey, J. E. and Kellerhals, M., *Mol. Breed.*, 2009, **24**, 337–347.
7. Dar, M. S. *et al.*, *Plant Pathol. J.*, 2015, **14**(4), 196–201.
8. Abe, K., Iwanami, H., Kotoda, N. and Moriya, S., *Plant Breed.*, 2010, **129**(2), 208–218.
9. Lechowicz, M. J., *Am. Nat.*, 1984, **124**, 821–842.
10. Primack, R. B., Laube, J., Gallinat, A. S. and Menzel, A., *Ann. Bot.*, 2015, **116**(6), 889–897.
11. Carisse, O., Jobin, T. and Bourgeois, G., *Can. J. Plant Sci.*, 2008, **88**(1), 229–238.

Received 15 December 2017; revised accepted 2 January 2019

RAMEEZ NAZIR RATHER[1,*]
AIJAZ A. WANI[1]
MEHRAJ-UL-DIN SHAH[2]

[1]*Cytogenetics and Reproductive Biology Laboratory,*
Department of Botany,
University of Kashmir,
Srinagar 190 006, India
[2]*Plant Virology and Molecular Pathology Laboratory,*
Division of Plant Pathology,
Sher-e-Kashmir University of Agricultural Sciences and Technology-Kashmir, Shalimar,
Srinagar 190 025, India
**For correspondence.*
e-mail: rameez674@gmail.com