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URDBEAN LEAF CRINKLE VIRUS: CURRENT SCENARIO AND FUTURE PROSPECTS

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A B S T R A C T

Urdbean leaf crinkle virus (ULCV) is a ubiquitous virus having colossal losses in urdbean and mungbean that spreads via all possible ways of virus transmission in Urdbean. ULCV causes variable losses that may range from 35- 94%, in mungbean and urdbean depending on host genotypes, by damaging and halting the plant growth at any stage. In infected plants, change in histopathological, physiological and biochemical behavior that results in high sugar contents, amino acid contents, total protein contents, phenol contents, increase in peroxidase activity with the decrease in chlorophyll contents, and superoxide dismutase activity. The development of ULCV disease has a direct correlation with favorable environmental factors, such as 35-42 ^{o}C as the maximum temperature and 21-29 ^{o}C as the minimum temperature. Whereas, wind movement, rainfall and relative humidity have no impact on disease progression. The management of ULCV is possible by adopting various approaches like the selection of healthy seeds, seed treatment, screening of germplasm, induced/ acquired systemic resistance in host, and by application of balanced nutrients to the plants. In the future, the work should be done on the molecular characterization of this virus to devise an effective and accurate management plan.

Keywords: Urdbean, Germplasm, Virus, Resistance, Management.

INTRODUCTION

Urdbean leaf crinkle virus (ULCV) is a prevalent, damaging, and commercially important pathogen inducing infection in black gram that results curling, crinkling, puckering, and yield reductions (Ashfaq *et al*., 2007). It causes major yield losses in mungbean (*Vigna radiata*) and urdbean (*Vigna mungo*) wherever they are grown. Urdbean is the most susceptible to ULCV than other pulses and can be passed on from diseased to healthy plants via grafting, sap inoculation, and seed (Gautam *et al*., 2016). It is a seed-borne pathogen with narrow host range. Yield losses in urdbean at different stages of crop due to urdbean leaf crinkle virus adversely affect growth parameters both in the field as well as artificially inoculated in the glasshouse (Bashir *et al.,* 2005).

Origin, Distribution and Taxonomy: Urdbean commonly known as black gram (Chandel, 1984) and is a short duration self-pollinated leguminous crop that belongs to the family *Fabaceae*. Lukoki *et al*. (1980) indicated Central Asia and India its origin having wide adaptation to subtropical areas (Ganguly and Bhat, 2012). Urdbean is important pantropical crop cultivated in Europe, Australia, America and Africa. Among people of South Asia, Sri Lanka, Bangladesh, India, and Pakistan are conventional development sites of black gram (Rubatzky and Yamaguchi 2012). Production of black gram is 7.5 thousand tonnes with cultivation of15.2 thousand ha in Pakistan according to Pakistan Economic Survey (2017-2018). Mostly urdbean varieties seed are black colored, lack sulphur processing amino acids but rich in proteins also have lysine and phosphoric acid. ULCV is a common, widely distributed and harmful pathogen causing leaf crinkle disease in Pakistan and also in India (Bashir and Zubair, 1985; Ilyas *et al,* 1992) (Figure 1). First time this disease was reported in Indian Punjab, termed as 'curly top' and later observed in States of Uttar Pradesh, Delhi and Terai region of Uttar Pradesh chronically (Chohan and Kalia 1967; William *et al*, 1968). In Pakistan ULCV

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was first reported in 1987 in infected seeds (Bashir *et al*, 1991). The main sources of disease transmission are seeds and insects i.e. pea aphid (*Acyrthosiphon pisum*) and lady bird beetle (*Henosephilachna dodecastigma*) including several species of aphid and whitefly (Nene, 1972; Kolte and Nene, 1972; Narayansamy and Jaganathan, 1973; Dhingra, 1975; Beniwal and Chaoubey, 1979; Beniwal and Bharathan, 1980; Dhingra and Chenula, 1981; Kadian, 1982; Chowdhury and Nath, 1983; Beniwal *et al,* 1984; Bhardwaj and Dubey, 1986; Brar and Rataul, 1987; Bashir *et al,* 2005). Host range of ULCV is restricted to leguminous family; whereas grafting, sap transmission, several weeds i.e. field bindweed (*Convolvulus arvensis*), Pigweed (*Trianthema monogyna*), jimson weed (*Datura stramonium*, *Datura metel*, *Datura metaloides*, *Datura incrimis*) and plant species i.e. hyacinth bean (*Dolichos lablab*), common bean (*Phaseolus vulgarus*), globe amaranth (*Gomphrena globosa*), chaff flower (*Achyranthes* spp.), amaranthus (*Amaranthus* spp.) punarnava (*Boerhavia diffusa*), marijuana (*Cannabis sativa*), plumed cockscomb (*Celosia argentea*), bathu (*Chenopodium amaranticolor*), quinoa (*Chenopodium quinoa*), nut grass (*Cyperus rotundis*), asthma plant (*Euphorbia hitra*), coral heath (*Epacris microphylla*), tobacco (*Nicotiana plumbaginifolia*), stone breaker (*Phyllanthus niruri*), milkworts (*Polygala chinensis*), red root (*Portulaca oleracea*), mungbean (*Vigna radiate*), mothbean (*Phaseolus aconitifolius*), bottlegourd (*Lagenaria 206ylindrical*), cucumber (*Cucumis sativus*), Clusterbean (*Cyamopsis tetragonoloba*), Redgram (*Cajanus cajan*), Groundnut (*Arachis hypogaea*), cocklebur (*Xanthium strumarium*) and soybean (*Glycine max*) are the hosts of ULCV (Gupta, 1974; Bhaktavatsalam, 1983; Beniwal *et al*, 1983; Kolte and Nene, 1975; Witter *et al*., 1980; Kadian, 1983; Kadian, 1994; Bashir *et al*, 2005).

Figure 1. Distribution of Urdbean leaf crinkle virus (ULCV). Source https://www.plantwise.org/KnowledgeBank/datasheet/55743

Virus Transmission, Symptomatology and Epidemiology: The Transmission of ULCV is through seed, grafting, insect vectors and plant sap. The disease primarily spread through infected seed whereas insect vectors are the secondary source of dispersal and help for multiplication. The Urdbean leaf curl disease etiology was first suspected by the William *et al*. (1968). However, the nature of virus infection was studied by Nene (1972). The work was supported by various researchers (Nene, 1972; Kotle & Nene 1972; Narayansamy & Jaganthan, 1973). Insect vectors i.e., leaf feeding beetle, whitefly and aphid can easily transmit the virus under the field conditions (*Henosepilachna dodecastigma* (Wied); Beniwal & Bharathan, 1980; Narayansamy and Jaganthan, 1973;

Dhingra, 1975; Dubey *et al*., 1983; Nath *et al*., 1986). Various studies on the rapid inoculation method of ULCV using germinated seed have been also reported by workers (Chowdhury and Nath, 1983; Ashok *et al.,* 1994; Ahmad *et al.,* 1997; Kolte and Nene 1972; Pushpalatha *et al*., 1999; Ravinder *et al.,* 2005). The most common ULCV signs include wavy appearance of leaf, lamina crinkling, and shortening of the petioles, resulting in leaf crowding. Sepals also become greener and thicker than normal whereas the leaf area of the diseased trifoliate enlarge than that of healthy ones (Brar and Rataul, 1986). Urdbean leaf crinkle disease develops at a maximum temperature of 35 ^{o}C (±2 ^{o}C), minimum temperature of 25 ^{o}C (± 2 ^{o}C), minimum relative humidity above 70% and evening relative humidity <50% (Ashfaq *et al*., 2008). In summer, the symptoms remain masked at 38-45 ^{o}C when morning and evening relative humidity remained 60 and 40%, respectively. The disease symptoms did not appear even at 35 ^{o}C or a week or above 47 ^{o}C for a day with morning and evening relative humidity of 45 and 20 %, respectively (Kadian, 1989).

Losses caused by ULCV: ULCV infection initiating at different stages of plants adversely affect the various growth parameters of urdbean (Bashir *et al*., 1991) resulting in yield losses. Sravika *et al*. (2018) reported significant reduction in the number of pods, plant height, number of seeds in plants those get infected earlier. About 45.09% reduction in plant height and 44.57% reduction in number of trifoliate leaves/plants was recorded. The losses in yield due to ULCV infection were also recorded by Kolte (1971); Nene (1973); Singh (1980); Kadian (1982) and Subba Rao (1984). Due to reduction in plant height and number of trifoliate leaves/plants there is a reduction in the synthesis of food materials and it also depends on the age of infection and cultivar. The nature of disease is systemic because with reduction in pod and seed yield there will be poor flowering and sterility of the inflorescence. Bashir *et al*. (1991) reported that 8% reduction in plant height, 90% decrease in pods/ plant and 26% decrease in the number of seeds/pod. The average yield loss of 81% was calculated when results were compared with uninfected plants. Beniwal and Chaubey (1979) observed decreasing in the number of pods and seeds/pods in urdbean; whereas, Narayanaswamy and Jaganathan (1973) and Ravinder (1988) also observed pollen sterility of plant depending on the age and at the time of infection. Bean common mosaic virus infection resulted in significant reduction in legume crops seed production. Ashok *et al*. (1994) also reported the reduction in the number of seeds per/ pod, seed weight and pod size in urdbean. Kadian, (1982) have also reported yield losses due to urdbean leaf crinkle disease ranged from 2.12% to 95.17% in in legume crop at Hissar in India. Chattopadhyay *et al*. (1986) and Manadhare *et al*. (1999) have also reported t yield losses due to ULCV varied from 14.6 to 55.8% in different cultivars of green gram and black gram.

Histopathological and biochemical changes: Biochemical changes occurred in mashbean due to leaf crinkle virus, sugar contents increase in the infected leaves compared with the healthy leaves, high amino acid contents changed in infected young leaves, at the early and late infection, respectively, while decreased in old leaves by 22.7 and 16.2 percent whereas as decrease in protein contents by 7.59 and 12.05 percent at earlier infection of young and old leaves, respectively and by 16.16 and 17.09 percent at the late stages of infection. The chlorophyll contents in infected young leaves were reduced but increased in old leaves (Brar and Rataul, 1990). Enlargement of leaves has also been reported due to increase level of indole acetic acid in leaves infected with ULCV (Bhaktavatsalam *et al*. 1983; Patel *et al*. 1999). Ravinder *et al.,* (2006) examined the anatomical changes in petioles and various leaves of parts of plants infected with ULCV, which were very visible and increased the number of layers and the size of parenchymal and epidermal cells and the thickness of the palisade layer and the spongy cell layer was more than healthy. The cross section of the ULCV-infected Urdbean stem showed a well-developed cortical layer three times thicker than that of the healthy leaf and number of vascular bundles were also increased. The number of vascular bundles was the same for healthy and infected petioles, but the number of rows of xylem vessels and vessels per row increased significantly for infected petioles. The infected xylem vessels (metaxylem and protoxylem) increased significantly compared to healthy plants. There was also a significant increase in the number of parenchyma and cambial cell layers and in the diameter of the phloem parenchyma cells in diseased plants compared to the healthy stems (Ravandar *et al*. 2006). Viruses have dependent metabolic system and they have to invade host cells to obtain the energy for biosynthesis (Kumagai *et al*., 1995). Reduction in catalase activity in virus infected plants was also reported by various researchers

(Srivastava and Singh 2010; Chen *et al*. 1993; Neuenschwander *et al*. 1995; Clarke *et al*. 2002), while Ashfaq *et al*. (2010) observed an increase in activity of catalase in ULCV infected plants. Results in other crops, e.g., broad bean infected with white clover mosaic virus (WClMV) and tobacco plant infected with TMV, also revealed reduction trend in catalase activity (Clarke *et al*. 2002; Chen *et al*.1993; Neuenschwander *et al*.1995) whereas an enhancement in catalase activity has also been reported by Riedle-Bauer (1998) and Hernandez *et al*. (2001).

Total Soluble proteins and Phenols: Ashfaq *et al*., 2010 reported higher contents of total protein in plants and highlighted this was due to the large number of proteins in both resistant and vulnerable plants at 15 and 30 days after inoculation. The study was supported by Srivastava and Singh (2010) in which they calculated a significant intensification in soluble protein contents in both susceptible and resistant cultivars at 10, 20 and 30 days post inoculation of virus. These results are in agreement with several workers (Shukla & Rao, 1994; Langhams & Glover, 2005; Yardimci *et al*., 2007). A decrease level of total soluble protein contents in plants infected with ULCV according to several researchers (Brar and Rataul, 1990; Thind *et al*., 1996; Taiwo & Akinjogunla, 2006). Increase in phenol contents in susceptible cultivars of urdbean due to ULCV has been reported as a failed attempt at resistance on the part of host (Karthikeyan *et al*., 2007; Rahioui *et al*., 2013; Ashfaq *et al*., 2014;).

Superoxide dismutase and Peroxidase: Level of superoxide dismutase (SOD) significantly decreases in resistant plants; whereas as results of SOD level in susceptible plants were reported non-significant by Ashfaq *et al*. (2010). Similar results of decrease in SOD level in different plant inoculated with different viruses were reported by various researchers (Riedle-Bauer 1998); Clarke *et al*. 2002; Hernandez *et al*. 2004). Ashfaq *et al*. (2010) observed higher level of peroxidase (PO) in healthy leaves than in the resistant plant. With the passage of time, activity of PO increased in both susceptible and resistant but after 15-30 days of inoculation. The increase in PO may be responsible for resistance mechanism in virus-infected plants. Similar results were reported by various other researchers (Nadlong and Sequeira, 1980; Van Loon, 1976; Clarke *et al*. 2002; Karthikeyan *et al*. 2007). Ashfaq *et al*. (2010) observed that increased peroxidase levels disrupt signals of enhanced ROS, and then it provides resistance against ULCV black gram.

Management of ULCV: Seed Treatment: Seed treatment with fungicide has been reported very effective method in controlling leaf crinkle disease of urdbean caused by ULCV. Drenching of 1% Benomyl (fungicide) in mungbean before inoculation of virus prevented the symptoms development significantly. The treatment at lower concentration reduced the virus transmission; whereas the drenching after virus inoculation proved less effective (Bhardwaj *et al*., 1982). Sharma and Dubey (1984) concluded that after drenching with 2% carbendazim/ Benomyl aphids were failed to acquire virus from infected plants. At 55 ^{o}C for 30 min hot water treatment eliminated the seed-borne infection of ULCV (Sharma and Dubey, 1984).

Screening of germplasm: Sravika *et al*. (2018) screened different urdbean cultivars against ULCV. He reported the use of resistant cultivars a very economical and ecofriendly approach against ULCV. The resistance was evaluated by less incidence, low spread and milder symptoms in resistant cultivars as compared to susceptible cultivars. A five and six-point disease severity scale was used to evaluate different blackgram and greengram cultivars/ lines under both field and artificial inoculated conditions (Beniwal and Chaubey, 1984; Subba, 1984; Vijay, 1993; Venkata, 1996; Lokesh, 1997; Rao, 2002;). Iqbal *et al*., (1991) stated that use of virus free seed and resistant genotypes of blackgram would be useful to check further spread in new blackgram/pulses growing areas. Bashir *et al*. (2006) concluded after a survey in major Urdbean growing areas that there is a lack of resistance in genotypes screened against ULCV in the fields. There is no availability of virucide, hence the identification of resistant varieties is the most useable and reliable method for the control of ULCV (Akhtar *et al*., 2011). A number of scientists has worked on screening of Urdbean germplasm to find out resistant source against *Mungbean yellow mosaic virus* (MYMV) (Sandhu *et al*., 1988; Malik, 1991; Naqvi *et al*., 1995; Singh *et al*., 1996; Varma *et al*., 1998; Singh *et al*., 2000; ; Singh *et al*., 2000; Khattak *et al*., 2003; Amavassai *et al*., 2004; Pathak and Jhamaria, 2004; Bashir *et al*., 2005; Bashir *et al*., 2006; Biswas *et al*., 2005; Shad *et al*., 2006; Akhtar *et al*., 2009 Khan *et al*., 2012; Ahmad *et al*., 2013; Sudha *et al*., 2013)

A large number of molecular techniques have been developed. Molecular characterization of genotypes using different molecular markers i.e., RAPD, SSR, ISSR and

SCAR have been tested by different workers to assess genotype diversity. Genotypes selected from different reaction groups in response to viruses are subjected to genetic analysis to show different level similarity and diversity. These markers could be used for the development of virus resistant cultivars Binyamin *et al*., (2011) stated that RAPD-based analysis could be used for cultivar identification in Urdbean. PCR technology has contributed to the development of a number of molecular test systems that can be used to detect polymorphisms. Advances in molecular biology have enabled plant breeders to effectively select from gene pools based on DNA markers and to separate populations. Molecular markers are used to successfully identify various agronomic traits (Hearnden *et al*., 2007; Azmat and Khan, 2010) and disease resistance genes (Michelmore *et al*., 1991). Several researchers have also used these molecular techniques in urdbean and mungbean (Santalla *et al*., 1998; Lakhanpaul *et al*., 2000; Ghaffor *et al*., 2001; Souframanien *et al*., 2002; Souframanien and Gopalakrishna, 2004; Dikshit *et al*. 2007; Lavanya *et al*., 2008; Arulbalachandran *et al*., 2009; Saini *et al*., 2010; Datta *et al*., 2012; Karthikeyan *et al*., 2012; Dhole and Reddy, 2013).

Characterization of environmental factors: The impact of environmental conditions on Urdbean leaf crinkle disease development have shown overall significant correlation with maximum and minimum temperature and no correlation with relative humidity, rainfall and wind speed. To characterize the environmental conditions conducive for the disease epidemics, it was found that maximum ULCV disease severity/incidence was recorded at 35-42 °C (maximum temperature) and 21-29 °C (minimum temperature). Kadian (1989) reported that 70% relative humidity is favorable for infection. The correct interpretation of disease-specific epidemiological data requires information about the occurrence of diseases in the past and in the present. The increase in the number of reported cases, and which is a seasonal pattern of change in host vulnerability. The weather is one of the most important parameters influencing the epidemics of plant diseases. It is therefore necessary to understand climate conditions in order to provide basic information for the development of simple and reliable disease forecasting systems. Only limited work has been carried out on the environmental conditions favorable to the development of ULCV.

Induction of systemic resistance and antiviral role of plant extracts and chemicals: Systemically induced resistance activates the immune system in the host with the development of virus inhibitors (VIA) (Verma and Dwivedi, 1984; Khan and Verma, 1990). The use of plant kingdom antiviral principles (AVPs) was considered the best alternative to increasing plant resistance to viral diseases (Narayanasamy and Ramiah, 1983; Narayanasamy, 1984; Verma and Khan, 1985; Rao *et al*., 1985; Ragupathi 1995; Kandan *et al*. 2002). AVPs affect virus replication and block mRNA activity (Owens *et al*. 1973). Proteins present in various plants have been described as ribosome inactivating proteins (RIPs) which interfere with virus propagation through process of ribosome inactivation (Balasaraswathi, 1995; Bolognesi *et al*., 1997; Vivanco *et al*., 1999). Urdbean inoculated with leaf extracts from *M. Jalapa*, *B. spectabilis* and *P. chilensis* showed a significant increase in the activity of phenyalanine ammonia lyase, peroxidase (PO) and polyphenol oxidase. PAL catalyzes the conversion of L-phenylalanine to trans-cinnamic acid in the first step of the phenylpropanoid pathway, which provides the lignin, UV protection agents (Hahlbrock & Grisebach 1979; Vance *et al*. 1980; Grisebach 1981; Hahlbrock & Scheel 1989; Lewis & Yamamoto 1990). Phenylpropanoid metabolism's activation is believed to result in the deposition of phenolic polymer, which creates new mechanical barriers against the invasion of pathogens (Favali *et al*. 1978). PO is involved in a number of plant defense mechanisms (Mareschbacher *et al*. 1986) in which H_2O_2 is often delivered through an oxidative shock, a common occurrence in defense reactions (Dixon & Lamb 1990). Plant cell walls tend to be a peroxidase reaction like lignification (Hammerschmidt *et al*., 1982), suberization (Espelie *et al*., 1986), and structural cell wall protein cross-linking (Fry 1986). The POPPO-H_2O_2 mechanism is used by both PO and PPO to catalyze the oxidation (Srivastava, 1987). The phenols are oxidized by PPO and PO into more toxic phenol derivatives (Hammerschmidt & Kuc, 1982). PPO catalyzed the monophenols hydroxylation to odiphenols and the dehydrogenation of o-diphenol (Vaughn & Duke 1984).

Studies have confirmed the antiviral role of some plant species (aloe vera, milk weed (Ak), great bougainvillea, onion and garlic) against ULCV with significant reduction in disease incidence (20-30% over the control) and delaying in symptom appearance up to two weeks. Some chemicals like Acetyl salicylic acid have potential to

suppress the disease incidence up to 80% over the control. Whereas, Carbendazim (0.2%) is also potent against ULCV (Ashfaq *et al*. 2006).

Role of nutrients: Nutrients play vital role in the management of ULCV (Zeshan *et al*., 2012) and it is also reported that plant defense mechanisms are regulated by the growth regulators (Jalali, 2005). Contribution of nutrients like N, P, K, Zn, B, Planofix (NAA) in the management of plant viruses as well as ULCV have also been acknowledged by some scientists (Islam *et al*., 2002; Ashfaq *et al*., 2014).

Future Directions: Urdbean leaf crinkle virus is an ungrouped virus and no molecular work has been done on this virus till now. Hence for future studies it is recommended that research should be conducted on molecular characterization of this virus, so that taxonomic position of this virus could be defined. As for accurate detection of this virus and developing measurement strategies molecular data and taxonomic position of plant virus is basic need. The above review clearly indicates that the knowledge on the molecular characteristics of ULCV, genetic variability, host-virus interaction and mechanism of its evolution and adaptability to different pulses is prerequisite to develop control strategies.

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Muhammad Ashfaq	:	Write, review and edited manuscript.
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