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ANTIXENOTIC RESISTANCE TRAITS OF MUSKMELON (*CUCUMIS MELO* L.) AGAINST FRUIT FLY (*BACTROCERA CUCURBITAE* (COQUILLETT)) IN ARID REGION OF INDIA

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ABSTRACT

Plant genotypes possess different antixenotic properties, which resultantly induce in them different mechanisms of resistance. Various antixenotic traits including length of pubescence, fruit toughness, rind thickness, flesh thickness, days to first harvest and fruit diameter were studied on eleven genotypes of muskmelon (*Cucumis melo* L.) in relation to resistance against *B. cucurbitae* under field conditions in the hot arid region of India. Significant differences were observed in the tested genotypes for fruit infestation and larval density per fruit. AHMM/BR-1, RM-50 and AHMM/BR-8 were resistant; MHY-5, Durgapura Madhu and Pusa Sarabati moderately resistant; AHMM/BR-13, Pusa Madhuras and Arka Jeet susceptible; while, Arka Rajhans and GMM-3 were the highly susceptible genotypes to fruit fly infestation. Spositive correlation (r = 0.97) was observed between per cent fruit infestation and larval density per fruit. The percent fruit infestation and larval density had significant positive correlations with fruit diameter and days to first harvest and negative correlation with fruit toughness, rind thickness, flesh thickness and length of ovary pubescence. Maximum variation in fruit infestation and larval density was explained by length of ovary pubescence (63.3 and 45.7% respectively) followed by fruit toughness (6.7 and 13.7% respectively) and fruit diameter (8.6 and 10.5% respectively).

Key words : *Bactrocera cucurbitae*, *Cucumis melo*, antixenotic, allelochemical, muskmelon

Musk melon (*Cucumis melo* L.) is one of the important horticultural crops worldwide and plays an important role in international trade. Different forms of melon are known that are morphologically different. It is a species of melon that has been developed into many cultivated varieties. These include smooth skinned varieties such as honeydew, crenshaw and casaba and different netted cultivars (cantaloupe, Persian melon and Santa Claus or Christmas melon). The main plant organ used is the fruit, which is used both immature and mature (McCreight and Staub, 1993) as desserts and vegetables for salad. Melon seeds may be eaten after being slightly roasted or edible oil can be extracted from them. Plants are generally exposed to a variety of biotic and abiotic factors that may alter their genotypic and/or phenotypic properties resulting in different mechanisms of resistance which enable plants to avoid, tolerate or recover from the effects of pest attacks (Sarfraz *et al.*, 2006; Gogi *et al.,* 2010a). Such mechanisms of plant resistance have been effectively used against insect pests in many field and horticultural crops (Dhillon *et al.,* 2005b; Sarfraz *et al.,* 2006; Sarfraz *et al.,* 2007; Gogi *et al.,* 2010b). Antixenosis, refers to the potential plant-characteristics/traits or morphological features that impair or alter insect behavior towards host preference (Afzal *et al*., 2009; Moslem *et al*., 2011, War *et al*., 2012, Haldhar *et al*., 2013), in such a way, as to lessen chances of insects, using a host plant for oviposition (Karban *et al*., 1997) food, damage or shelter (War *et al*., 2012).

Insect pests are a major constraint to increasing the production and productivity of muskmelon crop. The melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is a serious pest of muskmelon in India and its outbreak cause substantial crop losses to growers. The melon fruit fly has been observed on 81 host plants, but muskmelon is one of the most preferred hosts and has been a major limiting factor in obtaining good quality fruits and high yield (Nath and Bhushan, 2006). The extent of losses varies between 30 and 100 per cent, depending on the cucurbit species and the season. As the maggots damage the fruits internally, it is difficult to control this pest with insecticides; hence, development of varieties resistant to melon fruit fly is an important component of integrated pest management (Panda and Khush, 1995). Cultivation of genotypes resistant to fruit fly is a crucial component of integrated pest management programmes for muskmelon because of difficulties associated with chemical and biological control. Development of muskmelon genotypes resistant to fruit fly has been limited in India owing to inadequate information on the sources of plant traits associated with resistance to pest infestations. The present study was designed to identify various morphological (antixenotic mechanism) fruit traits of muskmelon genotypes associated with resistance against melon fruit fly in terms of fruit infestation and larval density under field conditions.

MATERIALS AND METHODS

Preliminary screening of muskmelon genotypes (summer, 2011). Twenty four genotypes of muskmelon viz., AHMM/BR-1, AHMM/BR-8, RM-50, MHY-3, MHY-5, Durgapura Madhu, Pusa Sarabati, AHMM/BR-3, AHMM/BR-15, AHMM/BR-14, Pusa Madhuras, AHMM/BR-32, Hara Madhu, Punjab Sunhari, AHMM/BR-25, AHMM/BR-35, AHMM/BR-7, Kashi Madhu, AHMM/BR-4, AHMM/BR-13, RM-43, Arka Jeet, Arka Rajhans and GMM-3 were sown at the experimental farm of Central Institute for Arid Horticulture (CIAH), Bikaner (at 28°06'N latitude, 73°21'E longitude and altitude of 234.84m above sea level). Seeds of muskmelon crop were soaked in water for two hours to soften their seed coat. The crop was sown during February, 2011 with three replicates for each genotype following a randomized block design. The area of each bed was 5 m × 2 m and the plant to plant distance was maintained at 50 cm with drip irrigation system. All the recommended agronomic practices (e.g. weeding, fertilization, hoeing, etc.) were performed equally in each experimental bed. Four pickings were done for the entire growing season of muskmelon fruits. The mean monthly minimum temperature and RH of both the years varied from 18.6- 27.9^{0}C and 14.7- 24.7 per cent, respectively. The maximum temperature and RH ranged from 32.9-42.2^{0}C and 45.6-56.5 per cent, respectively. Ten fruits were randomly selected from each picking from each experimental bed; a total of 30 fruits were taken from each picking of each genotype and were brought to the laboratory for microscopic examination for fruit infestation. The infested fruits were sorted and the percent fruit infestation was calculated. Ten fruits from all infested fruits from each picking of each genotype were then randomly selected for further examination, and the numbers of larvae were counted in each infested fruit. The genotypes were categorised by following the rating system given by Nath (1966) for fruit infestation as: immune (no damage), highly resistant (1–10%), resistant (11–20%), moderately resistant (21–50%), susceptible (51–75%) and highly susceptible (76–100%).

Final screening of the selected muskmelon genotypes(rainy, 2011 and summer, 2012). Eleven selected genotypes from preliminary screening of muskmelon, *viz*., AHMM/BR-1, AHMM/BR-8, RM-50, MHY-5, Durgapura Madhu, Pusa Sarabati, Pusa Madhuras, AHMM/BR-13, Arka Jeet, Arka Rajhans, GMM-3 were sown at experimental farm of CIAH, Bikaner in July, 2011 and February, 2012 following a RBD, with three blocks for each genotype and each block represented a replication. Agronomic practices were followed as described above.

Antixenotic fruit mechanism of the re-evaluated muskmelon genotypes. Ten marketable fresh fruits of each of the eleven genotypes were used to record data on the biophysical traits such as length of pubescence, fruit toughness, rind thickness, flesh thickness, days to first harvest and fruit diameter. Length of ovary pubescence, rind thickness, fruit diameter and flesh thickness were measured at five different positions of each fruit using Digital Vernier Caliper (MITU-TOYO, 300mm, 0.01mm reading capacity). The days of first harvesting of fruits was visually recorded in the field. The hardness of fruits was assessed at edible stage using fruit pressure tester (Model FT 327, 0-13kg/cm^2).

Statistical analysis. Transformations (angular and square root transformed value) were used to achieve normality in the data before analysis (Steel *et al.,* 1997); however, retransformed means are also presented in all the tables. The data on percentage fruit infestation, larval density per fruit and biochemical fruit traits were analyzed through one-way ANOVA using SPSS 16 software (O'Connor, 2000). The means of significant parameters, among tested genotypes, were compared using Tukey's honestly significant difference (HSD) tests for paired comparisons at probability level of 5 per cent. Correlations between biophysical and biochemical fruit traits and fruit fly parameters (percent fruit infestation and larval density per fruit) were determined using correlation analysis and backward stepwise multiple regression analysis at the 95 per cent significance level.

RESULTS AND DISCUSSION

Preliminary screening of muskmelon genotypes. Significant differences were observed in percentage fruit infestation and larval density per fruit among the tested genotypes during preliminary screening. The larval density per fruit had a significant positive correlation with percentage fruit infestation (r = 0.93; P < 0.01). AHMM/BR-1, RM-50 and AHMM/BR-8 were resistant; MHY-3, MHY-5, D. Madhu, P. Sarabati, AHMM/BR-3, AHMM/BR-15, AHMM/BR-14, P. Madhuras and AHMM/BR-32 moderately resistant; Hara Madhu, Punjab Sunhari, AHMM/BR-25, AHMM/BR-35, AHMM/BR-7, K. Madhu, AHMM/BR-4, AHMM/BR-

13, RM-43 and Arka Jeet susceptible and Arka Rajhans and GMM-3 highly susceptible genotypes. The larval density was highest in genotype Arka Rajhans (23.76 larvae/ fruit) followed by GMM-3 (22.64 larvae/ fruit). The minimum larval density was observed in AHMM/BR-1 (11.10 larvae/ fruit) followed by RM-50 (11.68 larvae/ fruit). The per cent fruit infestation was highest in Arka Rajhans (79.49 %) and lowest in AHMM/BR-1 (12.61 %) followed by AHMM/BR-8 (14.11 %). The fruit infestations ranged from 12.61 to 79.49 % and significantly lower in resistant genotypes and higher in susceptible genotypes.

Final screening of muskmelon genotypes. AHMM/BR-1, RM-50 and AHMM/BR-8 were found to be resistant; MHY-5, D. Madhu and P. Sarabati moderately resistant; AHMM/BR-13, P. Madhuras and Arka Jeet susceptible and Arka Rajhans and GMM-3 highly susceptible genotypes in both seasons (Table 1). The fruit fly infestation and larval density were higher during rainy season compared to summer season. The significant positive correlation ($r = 0.971$; $P < 0.01$) was observed between percent fruit infestation and larval density per fruit. Pooled data of larval density per fruit in both seasons (11.14–24.30 larvae per fruit) were significantly lower in resistant genotypes and higher in susceptible genotypes. The fruit infestation in rainy season ranged from 13.33 to 86.11 per cent and in summer season 12.63 to 79.52 per cent. Pooled data of fruit infestation in both seasons (12.98–82.81%) was significantly lowest in resistant genotypes and highest in susceptible genotypes. Pooled data for both seasons indicated that

Table 1. Larval density and per cent fruit infestation of fruit fly on different genotypes of musk melon during final screening trials

Genotypes	Larval density/ fruit (Pooled data)	Fruit infestation (%) (Pooled data)	Resistance category
AHMM/BR-8	12.43^{a}	15.72 (23.34)*^{a}	R
RM-50	12.06^{a}	15.49 (23.6)^{a}	R
AHMM/BR-1	11.14^{a}	12.98 (21.11)^{a}	R
MHY-5	15.05^{b}	30.14 (33.28)^{b}	MR
Durgapura Madhu	16.84^{bc}	37.41 (37.70)^{c}	MR
Pusa Sarabati	18.39^{cd}	46.52 (42.99)^{d}	MR
Arka Jeet	19.19^{d}	60.39 (51.00)^{e}	S
AHMM/BR-13	20.09^{de}	59.54 (50.48)^{e}	S
Pusa Madhuras	21.30^{ef}	50.08 (45.02)^{d}	S
Arka Rajhans	24.30^{g}	82.81 (65.53)^{f}	HS
GMM-3	23.11^{fg}	78.73 (62.52)^{f}	HS

*Values in parenthesis are angular-transformed. Value following different letter are significantly different using Tukey's HSD test, R- resistant, MR- moderately resistant, S- susceptible and HS- highly susceptible

Table 2. Antixenotic fruit traits of different genotypes of musk melon

Genotypes	Length of pubescence (mm)	Fruit toughness (Kg/cm^{2})*	Rind thickness (cm)	Flesh thickness (cm)	Days to first harvest*	Fruit diameter (cm)*
AHMM/BR-8	4.36^{de}	7.83 (2.97)^{d}	0.407^{e}	2.61^{bc}	72.53 (8.57)^{abc}	09.41 (3.22)^{a}
RM-50	5.06^{g}	8.93 (3.15)^{e}	0.293^{d}	2.50^{bc}	69.80 (8.41)^{ab}	09.63 (3.26)^{a}
AHMM/BR-1	4.78^{fg}	7.54 (2.92)^{d}	0.303^{d}	2.47^{bc}	70.07 (8.43)^{ab}	11.09 (3.48)^{ab}
MHY-5	3.61^{cd}	7.57 (2.93)^{d}	0.160^{ab}	2.21^{ab}	77.53 (8.86)^{d}	11.18 (3.49)^{ab}
Durgapura Madhu	3.98^{d}	6.10 (2.66)^{c}	0.147^{ab}	2.10^{ab}	68.40 (8.33)^{a}	10.38 (3.37)^{ab}
Pusa Sarabati	4.44^{ef}	6.40 (2.72)^{c}	0.170^{ab}	3.20^{c}	76.53 (8.80)^{d}	11.86 (3.58)^{bc}
Arka Jeet	2.28^{a}	4.07 (2.25)^{a}	0.140^{ab}	2.11^{ab}	78.40 (8.91)^{d}	09.69 (3.27)^{a}
AHMM/BR-13	3.58^{c}	6.17 (2.68)^{c}	0.187^{bc}	2.33^{abc}	76.00 (8.77)^{cd}	10.02 (3.31)^{a}
Pusa Madhuras	4.67^{efg}	5.10 (2.47)^{b}	0.127^{a}	3.11^{c}	79.00 (8.94)^{d}	11.86 (3.58)^{bc}
Arka Rajhans	2.78^{b}	6.73 (2.78)^{cd}	0.160^{ab}	1.54^{a}	78.20 (8.90)^{d}	13.51 (3.81)^{c}
GMM-3	2.35^{a}	4.74 (2.39)^{ab}	0.230^{c}	2.04^{ab}	74.27 (8.67)^{bcd}	11.92 (3.59)^{bc}

*Values in parenthesis are square root-transformed, Value following different letter are significantly different using Turkey's HSD test the per cent fruit infestation was recorded the highest in Arka Rajhans (82.81 %) and the lowest in AHMM/BR-1 (12.68 %) followed by RM-50 (15.49 %) (Table 1).

Antixenotic mechanism fruit traits of the re-evaluated muskmelon genotypes. The length of ovary pubescence, fruit toughness, rind thickness and flesh thickness ranged from 5.06 to 2.28 mm, 8.93 to 4.07 kg/cm^2, 0.407 to 0.127 cm and 2.61 to 1.54 cm, respectively, being significantly high in resistant and low in susceptible genotypes. The days to first harvest (68.40 to 79.00 days) and fruit diameter (9.41 to 13.51 cm) being significantly low in resistant and high in susceptible genotypes (Table 2). The length of ovary pubescence, fruit toughness, rind thickness and flesh thickness had significant negative correlations; whereas, days to first harvest and fruit diameter had significant positive correlations with the percentage fruit infestation and the larval density per fruit (Table 3). Stepwise regression analysis indicated that length of ovary pubescence, fruit toughness, rind thickness, flesh thickness, days to first harvest and fruit diameter explained 85.60 % of the total variation in fruit fly infestation. The maximum variation in fruit infestation was explained by length of ovary pubescence (63.3%) followed by fruit diameter (8.6%) and fruit toughness (6.7%) whereas, remaining of the biophysical fruit traits explained < 5.0% variation in the fruit infestation (Table 4). The length of ovary pubescence, fruit toughness, rind thickness, flesh thickness, days to first harvest and fruit diameter explained 82.9 per cent of the total variation in the larval density per fruit. The maximum variation in the larval density per fruit was explained by length of ovary pubescence (45.7%) followed by fruit toughness (13.7%), fruit diameter (10.5%), rind thickness (7.7%) and days to first harvest (5.2%) whereas flesh thickness

Table 3. Correlation coefficient (r) between percent fruit infestation and larval density per fruit with different antixenotic fruit traits of muskmelon genotypes

	Percent damage	Larva density	Fruit diameter (cm)	Fruit toughness (kg/cm2)	Rind thickness (cm)	Flesh thickness (cm)	Days to first harvest
Larval density	0.974^{**}						
Fruit diameter	0.586^{*}	0.636^{*}					
Fruit toughness	-0.717^{**}	-0.723^{**}	-0.204^{NS}				
Rind thickness	-0.599^{*}	-0.656^{*}	-0.440^{NS}	0.605^{*}			
Flesh thickness	-0.438^{NS}	-0.301^{NS}	-0.176^{NS}	0.086^{NS}	0.126^{NS}		
Days to first harvest	0.613^{*}	0.653^{*}	0.444^{NS}	-0.504^{NS}	-0.562^{*}	0.031^{NS}	
Length of ovary pubescence	-0.796^{**}	-0.676^{*}	-0.247^{NS}	0.656^{*}	0.393^{NS}	0.701^{**}	-0.466^{NS}

**Significant at P = 0.01 (two-tailed). * Significant at P = 0.05 (two-tailed)

Table 4. Backward stepwise regression models showing effect of different antixenotic fruit traits of muskmelon on larvae per fruit and percentage fruit infestation

Percent fruit infestation	R^2	Role of individual traits (%)
$Y= 7.56- 10.41X_1- 5.00X_2- 12.39X_3-2.99X_4+ 0.58X_5+ 6.86X_6$	85.60	8.60
$Y= 9.04- 6.23X_1- 5.44X_2- 35.44X_3- 11.84X_4+ 1.74X_5$	77.00	2.50
$Y= 130.79- 15.79X_1- 2.45X_2- 76.28X_3+ 2.48X_4$	74.50	0.10
$Y= 135.00- 14.42X_1- 3.05X_2- 74.59X_3$	74.40	4.40
$Y= 136.72- 14.37X_1- 5.78X_2$	70.00	6.70
$Y= 120.76- 20.01X_1$	63.30	63.30
Larval density per fruit		
$Y= -0.26- 0.30X_1- 1.42X_2- 2.86X_3- 1.38X_4+ 0.22X_5+ 1.39X_6$	82.90	10.50
$Y= 0.03+ 0.55X_1- 1.5X_2- 7.54X_3- 3.17X_4+ 0.46X_5$	72.40	5.20
$Y= 32.26- 1.98X_1- 0.72X_2- 18.35X_3+ 0.61X_4$	67.20	0.10
$Y= 33.30- 1.64X_1- 0.86X_2- 17.93X_3$	67.10	7.70
$Y= 33.71- 1.63X_1-1.52X_2$	59.40	13.70
$Y= 29.50- 3.12X_1$	45.70	45.70

X_1- length of pubescence, X_2- rind hardness, X_3- rind thickness, X_4- flesh thickness, X_5- days to first harvest, X_6- fruit diameter, R^2- coefficient of determination trait explained <1.0 per cent variation in larval density (Table 4).

Host plant selection by insects is either expressed by the occurrence of a population of insects on the plant in nature or by feeding, oviposition or use of the plant for complete offspring development (Rafiq *et al.,* 2008). Plants confront the herbivores both directly by affecting host plant preference or survival and reproductive success (direct defense), and indirectly through other species such as natural enemies of the insect pests (indirect defense) (Hower and Jander, 2008; Dudareva *et al*., 2006; Arimura *et al*., 2009). Direct defenses are mediated by plant characteristics that affect the herbivore's biology such as mechanical protection on the surface of the plants (e.g., hairs, trichomes, thorns, spines, and thicker leaves) that either kill or retard the development of the herbivores (Hanley *et al*., 2007). In the present study, AHMM/BR-1, RM-50 and AHMM/BR-8 were resistant and Arka Rajhans and GMM-3 highly susceptible genotypes of muskmelon against fruit fly. The percentage fruit infestation and larval density were found significantly lower in resistant genotypes and higher in susceptible genotypes of muskmelon. Numerous studies have shown that genotypes of the same species could significantly differ in their resistance to insect pests (Weems and Heppner, 2001; Sarfraz *et al*., 2006; Gogi *et al*., 2009; Gogi *et al*., 2010b; Moslem *et al*., 2011, Haldhar *et al.,* 2013) and it is caused by morphological traits of plants. Similarly, our findings also corroborate with that of Gogi *et al*. (2010b) and Dhillon *et al*. (2005a) who observed lower fruit infestation and larval densities on resistant genotypes of bitter gourd than on their susceptible genotypes.

The antixenotic mechanisms of fruit traits were significantly different among the tested muskmelon genotypes. Fruit diameter and days to first harvest had significant positive correlations; whereas, fruit toughness, rind thickness, flesh thickness and length of ovary pubescence had significant negative correlations with the percent fruit infestation and larval density. In these findings, biophysical fruit-traits were also found significantly different among genotypes (Dhillon *et al*., 2005a, Gogi *et al*., 2010a). Structural traits such as spines and thorns (spinescence), trichomes (pubescence), toughened or hardened leaves (sclerophylly), incorporation of granular minerals into plant tissues, and divaricated branching (shoots with wiry stems produced at wide axillary angles) play a leading role in plant protection against insects (Hanley *et al*., 2007; Chamarthi *et al*., 2010; He *et al*., 2011). Pubescence consists of the layer of hairs (trichomes) extending from the epidermis of the above ground plant parts including stem, leaves, and even fruits, and occur in several forms such as straight, spiral, stellate, hooked, and glandular (Hanley *et al*., 2007). Chamarthi *et al*. (2010) reported that leaf glossiness, plumule and leaf sheath pigmentation were responsible for shoot fly *Atherigona soccata* (Rondani) resistance in sorghum *Sorghum bicolor* (L.) (Moench). Similar results were documented by Gogi *et al*. (2010a) that fruit-length, fruit diameter, number of longitudinal ribs/fruit and number of small ridges/cm^2 that were significantly lowest in resistant genotypes but highest in susceptible genotypes had a significant positive correlation with the percent fruit infestation and larval density per fruit. Fruit toughness, height of small ridges, height of longitudinal ribs and pericarp thickness, which were significantly highest in resistant and lowest in susceptible genotypes, had a significant negative correlation with the percent fruit infestation and larval density per fruit. These variations in measurements of biophysical fruit-traits may be attributed to differences in the tested genotypes and/or stage of the fruits selected for measuring these traits, as reported in earlier studies (Dhillon *et al*., 2005a; Kumara *et al*., 2006; Gogi *et al*., 2010a).

Stepwise regression analysis indicated that maximum variation in fruit infestation was explained by length of ovary pubescence followed by fruit diameter and fruit toughness. The maximum variation in the larval density per fruit was explained by length of ovary pubescence followed by fruit toughness and rind thickness. As a result of limited literature on such aspects, present finding can be compared with the findings of only few researchers, who documented such interaction between plants or plant parts and insects other than that studied in the present research. Trichome (pubescence) density negatively affects the ovipositional behavior, feeding and larval nutrition of insect pests (Handley *et al*. 2005). In addition, dense trichomes affect herbivory mechanically, and interfere with the movement of insects and other arthropods on the plant surface, thereby, reducing their access to leaf epidermis (Agrawal *et al*., 2009). These can be, straight, spiral, hooked, branched, or un-branched and can be glandular or nonglandular (Honley *et al*., 2007). Glandular trichomes secrete secondary metabolites including flavonoids, terpenoids, and alkaloids that can be poisonous, repellent, or trap insects and other organisms, thus forming a combination of structural and chemical defense (Honley *et al*., 2007; Sharma *et al*., 2009). Structural defenses includes morphological and anatomical traits that confer a fitness advantage to the plant by directly deterring the herbivores from feeding (Agrawal *et al*., 2009) and range from prominent protrubances on a plant to microscopic changes in cell wall thickness as a result of lignifications and suberization (Hanley *et al*., 2007; He *et al*., 2011). Gogi *et al*., (2010a) indicated that the tested morphological traits explained 100 per cent of the total variation in fruit infestation and larval-density per fruit. The maximum variation, in fruit infestation and larval-density per fruit, was explained by fruit toughness followed by fruit-diameter and number of longitudinal ribs.

In summary, reduction in the fruit fly infestations on resistant genotypes could be due to antixenotics (biophysical) and our results suggest that biophysical fruit traits could contribute to these mechanisms of resistance. Certain biophysical traits (e.g. length of pubescence, fruit toughness and rind thickness) were linked to resistance of muskmelon against *B. cucurbitae* and therefore, can be used as marker traits in plant breeding programmes to select resistant genotypes.

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