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Hybrid Vigor for Alkaloid Contents in Opium Poppy (*Papaver somniferum* L.)

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ABSTRACT

This study aimed to determine suitable opium poppy hybrids over mid parent, better parent and standart check levels in alkaloid ratios. In the study, 36 hybrids were evaluated derived from nine varieties. The experiment was carried out in four replications in randomized block design in 2016 and 2017. Heterosis over mid parent values ranged from -4.91% to 65.43% for morphine, -76.91% to 278.05% for thebaine, -68.97% to 180.91% for noscapine, -53.60% to 150.45% for codeine, -93.55% to 289.61% for oripavine and -78.69% to 285.94% for papaverine. In the result study of 34 crosses recorded positive heterosis over mid parent for morphine content followed by 24 for thebaine, 23 for noscapine, 26 for codeine, 22 for oripavine and 15 for papaverine.

Key words: Alkaloid content, Diallel hybridization, Heterosis, Hybrid vigor, *Papaver somniferum* L., Poppy.

INTRODUCTION

Medicinal and aromatic plants have been used for human and animal health throughout history and they have always been beneficial in preventing and curing diseases. The increasing demand for natural products in recent years has led to the discovery of new uses of medicinal and aromatic plants, thereby booming the demand for these plants every other day. However, due to bad effects of synthetic chemicals on health, the significance of medicinal and aromatic plants, the active ingredient of many of the drugs used today is clearly seen. Poppy (*Papaver somniferum* L.), which has been used as a natural pain killer by human kind for centuries, is an important medicinal plant utilized by the pharmaceutical industry (Yazici, 2018). Opium poppy is an annual cultivar with 2n = 22. It belongs to *Papaver* genus from *Papaveraceae* family of Rhoedales group. There are 28 genera and about 250 species in the *Papaveraceae* family. Among them 7 species belongs to this family in Turkey. Studies report that there are a total of 58 papaver taxa including 36 species, 22 subspecies and varieties, 15 of which are endemic (Guner, 2012).

Breeders often use selection and introduction breeding, especially hybridization, to obtain genetic variation. Various methods such as diallel, partial diallel, or line x tester are used in the selection of parents in hybridization breeding. Hybrid vigor is defined as a measure of the superiority of F_1 hybrid progenies over their parents. This superiority can show itself in yield and quality, as well as in plant habitus and in increased endurance to extreme climatic conditions or diseases and pests. Heterosis over mid parent is defined as the difference between the average of two parents and their hybrids, while heterosis over better parent or heterobeltiosis is characterized as the difference between the superior parent and its hybrids.

The determination of heterosis and heterobeltiosis informs the breeder about which hybrid combination to choose and work on particularly and helps the breeder see the difference between the parent and its hybrids better (Korkut, 1981). The magnitude of heterosis provides information on extent of genetic diversity of parents and has direct bearing on the breeding methodology to be adapted for varietal improvement (Jawaharlal *et al.,* 2012; Thamodharan *et al.,* 2016). The aim of this study is to determine the appropriate crossbreeds by examining the hybrid progenies in terms of their heterosis over mid parent, better parent or heterobeltiosis and standart check levels in alkaloid ratios.

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MATERIALS AND METHODS

Nine different opium poppy varieties were used for half diallel hybridization. The varieties used in the trial were Ofis 96, TMO 1, Huseyinbey, Celikoglu, Ofis NM, Ofis 1, Bolvadin 95, Ofis 2 and TMO T. The variety Ofis 1 was used as the standard check. The genetic material of the study consisted of nine parents and 36 hybrids obtained by applying half diallel hybridization method [n. (n-1) / 2]. In order to obtain the hybrid seeds planned to make up the study material, 9 opium poppy cultivars used as a parent were sown in the experimental field of Middle Black Sea Crossing Belt, Directorate of Agricultural Research Institute under Tokat-

Kazova conditions on 27 February 2015. The varieties used in the trial were sown in three replications in two rows of 3 m in length with 45 cm row spacing and 10 cm plant spacing with later thinning by leaving 1 m space between varieties for the ease of performing hybridization and inbreeding operations. In order to make the blooming dates of the varieties to be used as father and mother coincide, sowing of all varieties was repeated in two rows one month later. F1 hybrid seeds obtained from hybridization and inbred parents were divided into two and they were sown in the trial field belonging to Middle Black Sea Crossing Belt Agricultural Research Institute Directorate in 2015-2016 and 2016-2017 vegetation period under Tokat-Kazova conditions in four replications according to randomized block trial design. The trial was carried out in two rows of 3 m in length with 45 cm row spacing and 10 cm plant spacing with later thinning.

In the study, adequate amount of samples were extracted from capsule shells of each combination, they were ground to powder and then their alkaloid (morphine, thebaine, noscapine, codeine, oripavine and papaverine) ratios were determined using HPLC analysis method (Kucuk, 1996).

The effects of hybrid vigor were determined by calculating separately as heterosis over mid parent and heterosis over better parent or heterobeltiosis. The estimates of heterosis over the mid parent, better parent (heterobeltiosis) and standart heterosis were calculated on the two years average.

Heterosis over mid parent

In terms of each characteristic investigated in the study, the relative increase (%) in the average of the F1 progeny generation to the parent average was calculated using the following equation (Chiang and Smith, 1967). The % heterosis was calculated from the formula: Heterosis over mid parent (HT) = 100 × (F1-MP) / MP, MP = Mid Parent (two varieties average is called MP). Heterosis over better parent or heterobeltiosis: In terms of each characteristic investigated in the study, the relative increase (%) in the average of the F1 progeny generation to the superior parent average was calculated using the following equation (Fonseca and Patterson, 1968). The % heterobeltiosis was calculated from the formula: Heterosis over better parent or heterobeltiosis (HB) = 100 × (F1-BP) / BP, BP = better parent / higher parent. Heterosis over standart check was calculated from the formula: SC = 100 x (F1-SC) / SC. The test of significance was carried out following "t" test for heterosis over the mid parent, better parent and standart check (Gami and Chauhan, 2013).

RESULTS AND DISCUSSION

Morphine and thebaine

Hybrid vigor values were measured in opium poppy hybrids. According to the average findings of two years, heterosis over mid parent values for morphine content ranged from -4.91% to 65.43% and with average 19.67%, heterobeltiosis ranged from -35.46% to 41.41% and with average -5.47%. Standart heterosis ranged from -42.71% to 109.65% and with average 20.24% (Table 1). Heterosis over mid parent was found the highest in TMO 1 x Bolvadin 95 hybrid and the lowest in Ofis 1 x Ofis 2 hybrid. Heterobeltiosis was the highest in TMO 1 x Bolvadin 95 and the lowest in Ofis 96 x Ofis 2 hybrids. Standart heterosis was the highest in Ofis NM x Ofis 1 and the lowest in Ofis 96 x Hüseyinbey hybrids.

In the study, in 34 hybrid combinations exhibited positive heterosis over mid parent for morphine content and fifteen hybrids recorded significant positive value over mid parent. Earlier studies reported Valizadeh *et al*. (2017). Among the 42 reciprocal hybrids of poppy heterosis was seen in positive direction for 40 of them such that 2 of them were observed in the negative direction. Singh and Pandey (2011), hybrid BR 233 x BR 242 exhibited the highest magnitude of positive significant sca effects with high positive heterosis over mid parent for morphine content. In general, there was no consistent association between sca effects and heterosis observed among the best hybrids identified.

Important amounts of heterosis have been observed for morphine, Gumuscu (2002) heterosis effect was investigated with 7 varieties of poppy and their reciprocal hybrids. According to the results of the research, heterosis was determined as -24.21% and 44.62% heterobeltiosis from -32.44% to 32.68%; in terms of morphine ratio. Dogramacı (2013) investigated the heterosis effect on yield and yield components of some poppy (*Papaver somniferum* L.) hybrids. When evaluated in terms of heterosis and heterobeltiosis; morphine rate heterosis effect ranged from -13.21% to 15.70%, heterobeltiosis from 26.98% to 11.11%. Khatik (2016) who carried out study in 8 poppy parents and their half diallel 28 F1 hybrids and 2 standard varieties in two locations. Heterosis varied from -9.97% to 10.03%, heterobeltiosis value between -12.19% and 9.39% for morphine in opium.

Regarding the rate of thebaine over the average findings of two years, heterosis over mid parent values ranged from -76.91% to 278.05%, average 35.35%. Heterobeltiosis values ranged from -88.23% to 185.20% and with average -5.66% and the estimates standart heterosis for thebaine content ranged from -86.69% to 319.56% and with average 11.00% (Table 1). Heterosis over mid parent and heterobeltiosis were obtained in the highest combinations of Celikoglu x Ofis 1 and lowest TMO 1 x TMO T. Standart heterosis was the highest in Ofis 2 x TMO T and the lowest in TMO 1 x Hüseyinbey hybrids. In 24 hybrid combinations exhibited positive heterosis over mid parent for thebaine content and four hybrids recorted positive significant. Among hybrids, fourteen hybrids recorded positive significant over standard heterosis for thebaine.

Noscapine and codeine

For noscapine content, heterosis over mid parent values varied from -68.97 to 180.91% with average 44.21%, heterobeltiosis ranged from -83.90% to 124.49%, average

Table 1: Heterosis (MP), heterobeltiosis (BP) and standart check (SC) values (%) for morphine, thebaine and noskapine.

Hybrid	Morphine (%)			Thebaine (%)			Noskapine (%)		
	MP	BP	SC	MP	BP	SC	MP	BP	SC
1x2	20.4	4.3	-22.5	-59.4	-77.1*	-76.8	-48.0*	-66.8**	-21.9
1x3	1.5	-4.8	-42.7**	-73.1	-78.3	-74.4	-65.9**	-75.9**	-40.3
1x4	6.2	-7.2	-30.0*	-71.9	-80.7*	-83.8	-68.9**	-83.9**	-53.9
1x5	12.7	-13.8	-4.5	67.1	33.3	40.3	-57.1**	-69.6**	-18.9
1x6	20.2*	-22.1**	53.7**	45.8	19.8	16.6	44.1	14.5	167.3**
1x7	22.9	11.4	-30.9*	-35.3	-55.7	-54.7	-61.1**	-71.8**	-39.3
1x8	-4.0	-35.4**	7.5	-62.7	-75.0	-72.1	-57.93**	-75.1**	-36.3
1x9	17.2	-12.3	4.9	-41.5**	-64.9**	84.3	-57.38**	-68.2**	-5.7
2x3	6.0	-11.2	-33.6**	-60.7	-76.9	-86.7	-25.6	-35.2	-53.6
2x4	8.5	0.0	-23.8	83.4	34.7	-58.6	37.3	-16.1	-62.4
2x5	14.5	-7.9	2.0	59.4	-5.4	-49.3	-11.9	-33.2	-37.9
2x6	28.7**	-13.4*	69.6**	38.6	-19.2	-52.7	129.7**	57.1	94.19*
2x7	65.4**	41.4*	0.4	123.4	49.9	-46.3	54.1	19.1	10.8
2x8	23.4*	-13.4	46.5**	128.8	47.0	-35.5	144.1*	85.2	31.3
2x9	28.1*	3.0	21.2	-76.9**	-88.2**	-30.6	46.9	3.9	15.9
3x4	7.5	-4.9	-31.4*	36.7	0.7	-34.3	64.6	-4.7	-31.1
3x5	10.3	-17.8	-8.6	95.3	46.7	11.3	-5.2	-21.4	-32.9
3x6	13.4	-27.4**	42.7**	106.3	80.6	22.3	126.8**	76.2*	125.6**
3x7	28.0	21.2	-31.0*	18.8	-13.0	-41.4	-6.7	-15.6	-33.4
3x8	6.9	-29.0**	17.5	-38.7	-54.6	-75.6	30.7	3.1	-33.1
3x9	32.7*	-3.5	15.7	-30.9*	-61.4**	95.8*	61.4	29.6	68.5
4x5	7.6	-10.5	-0.2	115.5	47.0	10.6	49.0	-16.6	-25.2
4x6	26.1**	-13.5**	71.2**	278.0**	185.2*	82.4	153.9**	40.0	73.6
4x7	21.8	6.3	-22.8	11.1	-26.7	-59.2	68.5	-3.3	-27.6
4x8	10.5	-19.9**	32.9*	89.9	52.6	-39.5	125.1	38.2	-52.8
4x9	8.7	-10.8	5.5	27.37*	-32.1**	284.1**	-1.0	-43.0	-19.6
5x6	36.1**	6.7	109.6**	68.3	31.8	-8.3	168.3**	124.4**	209.9**
5x7	38.2**	2.4	14.5	59.1	16.9	-44.9	10.7	-6.7	0.8
5x8	40.5**	17.9*	96.4**	206.6*	120.6	36.4	157.9**	97.0*	90.7*
5x9	32.1**	20.4	51.6**	28.9	-27.7**	273.0**	40.1	12.7	61.2
6x7	27.7**	-18.5**	60.0**	100.8	42.4	-13.8	60.7	27.4	59.2
6x8	-4.9	-14.4**	72.8**	29.3	-5.0	-42.0	180.9**	82.4*	129.6**
6x9	23.0**	0.1	95.8**	22.4	-31.5**	251.6**	129.6**	96.9**	214.3**
7x8	0.5	-33.5**	10.5	-28.9	-52.0	-76.8	24.8	-8.9	-27.2
7x9	33.2*	-3.3	13.8	-55.1*	-76.0**	24.9	-31.4	-40.70*	-20.5
8x9	35.9**	17.1*	94.0**	67.1**	-11.2*	319.5**	180.2**	98.5**	184.6**
Mean	19.67	-5.47	20.24	35.35	-5.66	11.00	44.21	4.16	24.00
SE	0.09	0.10	0.10	0.04	0.05	0.05	0.04	0.04	0.04

*, **, Significant at 5% and 1% level, respectively. Hybrids: (1):Ofis 96, (2): TMO 1, (3):Huseyinbey, (4):Celikoglu, (5):Ofis NM, (6):Ofis 1, (7):Bolvadin 95, (8):Ofis 2, (9):TMO T, SE: Standard error.

4.16% and standart heterosis ranged from -62.44% to 214.38% in the average findings of two years. Positive heterosis over mid parent was recorded in 23 crosses for noscapine content and eight of these hybrids were found positive significant. For over better parent seventeen crosses shows positive heterosis and six crosses were found positive significant. The hybrids Ofis 1 x Ofis 2, Ofis NM x Ofis 1 and Ofis 1 x TMO T showed highest heterosis over mid parent, better parent and standart check for noskapine content respectively. The hybrid Ofis 96 x Celikoglu showed lowest heterosis over mid parent and heterobeltiosis and the cross TMO 1 x Celikoglu over standart check (Table 1).

Heterosis over mid parent values for codeine ratio varied from -53.60% to 150.45%, average 40.44%, heterobeltiosis from -63.47% to 120.64%, average 7.90% and standart heterosis ranged from -55.21% to 180.10% and with average 33.15%. Positive heterosis over mid parent was recorded in 26 crosses for codeine content followed in 18 crosses over better parent and in 27 crosses over standart check. The hybrids Ofis 1 x TMO T showed highest heterosis over mid

parent, better parent and standart check for codeine content wheras the crosses Ofis 96 x Ofis 2 showed lowest heterosis over mid and better parent and the cross Huseyinbey x Ofis 2 over standart check (Table 2).

Mishra *et al*. (2016), in four poppy hybrid lines, to determine yield and quality characteristics and genetic progression in three generations, morphine rate heterosis values ranged from -11.80% to 10.58%, heterobeltiosis from -13.55% to 3.76%, codeine rate heterosis varied from -47.50% to 18.92, heterobeltiosis from -60.06% to -21.67%, thebaine rate heterosis varied from -83.18% to 49.96%, heterobeltiosis from -84.26% to 33.65%, noscapine ratio heterosis varied from -100% to 1686%, heterobeltiosis from -100% to 793% have been reported and narcotine showed positive and highest values of heterosis over mid and better parent in the cross NBMHT-2 × NBMHT-1.

Oripavine and papaverine

Heterosis over mid parent values for oripavine content ranged from -93.55% to 289.61%, average 42.47%, heterobeltiosis varied from -96.76% to 200.34%, with

Table 2: Heterosis (MP), heterobeltiosis (BP) and standart check (SC) values (%) for codeine, oripavine and papaverine.

Hybrids	Codeine			Oripavine			Papaverine		
	MP	BP	SC	MP	BP	SC	MP	BP	SC
1x2	33.1	-3.2	14.8	46.4	-13.4	-86.9	210.5	120.4	-49.4
1x3	-6.0	-36.4	-6.5	-68.9	-84.1	-91.9	56.8	-2.8	97.5
1x4	-22.0	-31.6	-27.6	-64.2	-78.6	-87.5	-54.9	-65.0	-73.8
1x5	13.0	-17.8	34.4	53.2	-18.4	-19.4	-41.4	-69.9*	98.3
1x6	86.27*	38.5	79.58*	-54.2	-75.3	-84.4	-64.8	-77.9	-63.1
1x7	11.4	-19.4	8.9	4.8	-47.5	-93.8	-38.7	-67.9	-47.5
1x8	-53.6	-63.5*	-43.0	-20.9	-56.1	-92.5	16.0	-29.3	-43.8
1x9	-13.4	-42.4*	20.2	-57.0**	-77.6**	166.9	135.8	78.4	118.2
2x3	25.3	17.0	-11.9	118.5	93.5	-79.4	105.3	42.4	100.6
2x4	18.4	-7.1	8.4	120.6	95.3	-52.5	150.2	80.5	5.6
2x5	-9.3	-29.3	1.0	109.0	41.2	-73.8	-14.7	-53.8	102.9
2x6	78.6	58.3	41.7	-2.7	-41.8	-85.0	-65.4	-79.7	-76.9
2x7	69.1	12.4	22.7	160.4	108.5	-49.4	99.5	36.1	65.0
2x8	28.0	-5.1	8.1	75.0	38.8	-90.0	200.1	114.2	108.4
2x9	40.8	23.4	25.1	-93.6**	-96.8**	-47.5	-57.1	-77.2	-46.9
3x4	77.48*	34.6	59.2	-38.5	-54.3	-91.3	125.4	75.2	120.4
3x5	-7.0	-34.0	-2.3	170.3	115.6	48.8	74.3	28.7	190.13**
3x6	121.17**	97.26*	74.06*	125.7	100.2	-45.0	185.76**	90.25**	200.88**
3x7	91.5	21.6	17.8	93.3	16.7	-89.4	19.5	-13.2	110.1
3x8	-31.8	-51.3	-55.2	81.4	22.9	-90.6	-5.1	-33.1	11.9
3x9	110.17**	64.2	106.15**	-72.3**	-86.1**	30.0	106.8	50.4	180.66*
4x5	-13.7	-29.2	14.2	130.3	105.9	3.8	97.9	-0.8*	59.4
4x6	147.71**	98.30**	128.96**	168.2	110.5	61.9	190.64*	95.3	168.2
4x7	53.7	-0.5	34.9	50.3	20.7	-81.9	285.9	145.5	61.3
4x8	40.4	11.8	-0.1	-35.4	-53.3	-92.5	-19.6	-47.3	-73.8
4x9	30.2	-0.6	60.8	12.3	-42.8**	250.25**	-78.7	-89.2	-23.8
5x6	80.53**	45.5	103.33**	-36.7	-53.6	-91.3	117.5	68.7	188.75**
5x7	4.5	-19.9	12.1	110.67	77.1	-72.5	91.2	19.3**	77.5
5x8	45.7	6.6	66.4	180.2	120.4	-2.5	175.78**	85.3	195.25**
5x9	57.58*	28.0	113.75**	-9.9	-53.6**	210.75**	98.6	76.7	170.63*
6x7	98.69*	42.5	53.3	289.6	200.3	10.6	-3.0	-33.4	82.5
6x8	-4.6	-27.3	-35.6	54.5	9.7	-81.9	92.6	29.6	20.0
6x9	150.45**	120.64**	180.10**	-22.2*	-60.1**	175.13**	-44.9	-61.7	16.3
7x8	-34.3	-53.0*	-43.8	16.7	-2.5	-92.5	-68.6	-84.0	-70.0
7x9	74.7	9.5	39.1	-76.8**	-88.3**	27.5	-64.8	-79.9*	-44.4
8x9	63.32*	25.6	90.31*	11.0	-44.2**	200.00**	-68.0	-78.5	-66.9
Mean	40.44	7.90	33.15	42.47	4.14	-18.87	54.06	5.34	51.95
SE	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.02

*, **, Significant at 5% and 1% level, respectively. Hybrids: (1):Ofis 96, (2): TMO 1, (3):Huseyinbey, (4):Celikoglu, (5):Ofis NM, (6):Ofis 1, (7):Bolvadin 95, (8):Ofis 2, (9):TMO T, SE: Standard error.

average of 4.14%. Standart heterosis varied from 93.75% to 250.25% and with average 18.87%. Heterosis over mid parent and heterobeltiosis were found highest in Ofis 1 x Bolvadin 95 hybrid wheras lowest TMO 1 x TMO T hybrid. The hybrid Celikoglu x TMO T showed highest heterosis over standart check for oripavine content wheras the hybrid Ofis 96 x Bolvadin 95 showed lowest heterosis over standart check (Table 2).

Heterosis over mid parent values of papaverine content ranged from -78.69% to 285.94%, average 54.06%, heterobeltiosis values ranged from -89.15% to 145.50% and with average 5.34%. standart heterosis ranged between -76.88-200.88% and with average 51.95%. The highest heterosis over mid parent and heterobeltiosis were obtained from the hybrid Celikoglu x Bolvadin 95 wheras the lowest the hybrid Celikoglu x TMO T. the hybrid Huseyinbey x Ofis 1 showed heterosis over standart check for papaverine content wheras the hybrid TMO 1 x Ofis 1 showed lowest heterosis over standart check (Table 2).

Khatik *et al*. (2017) reported that 16 hybrids recorded significant positive heterosis for latex yield per plant, nine and six hybrids recorded significant positive heterobeltiosis and economic heterosis for latex yield per plant, respectively.

CONCLUSION

In this study, were used nine parents and 36 hybrid combinations obtained in 2015 by half-diallel hybridization. The experiment was conducted in 2016 and 2017. F1 hybrid combinations were evaluated according to heterosis over mid parent, better parent and standart check values for alkaloid content on the average findings of two years. For morphine content 15 hybrids exhibited positive significant heterosis over mid parent, while 3 and 13 hybrids exhibited positive significant heterosis over better and check respectively. Positive significant heterosis over mid parent was recorded in 4 crosses for thebaine content followed 9 for noscapine, 10 for codeine, 3 for papaverine. The crosses TMO 1 x Bolvadin 95, Ofis NM x Ofis 1, Ofis NM x Ofis 2, Ofis NM x Bolvadin 95 for morphine; Celikoglu x Ofis 1, Ofis NM x Ofis 2 and Ofis 2 x TMO T for tebaine; Ofis 1 x Ofis 2, Ofis 2 x TMO T, Ofis NM x Ofis 1 and Ofis 1 x TMO T for noscapine; Ofis 1 x TMO T, Celikoglu x Ofis 1 and Hüseyinbey x Ofis 1 for codeine; Ofis 1 x Bolvadin 95, Ofis NM x Ofis 2 and Celikoglu x TMO T for oripavine; Celikoglu x Bolvadin 95 and Huseyinbey x Ofis 1 for papaverine were identified as superior hybrids. These crosses can be evaluated as suitable for breeding studies.

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