

枣规模化杂种创制技术体系的建立与应用研究进展

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摘要:建立枣树的规模化杂种创制技术体系,尽快培育出人工杂交优良新品种,满足枣生产中对杂交优良品种的迫切需要,对提高枣育种技术水平和促进枣产业发展意义重大。本文基于枣杂种创制实践,系统归纳总结了亲本的选择选配、蜜蜂辅助授粉技术、提高亲本结实率技术、后代培养及鉴定、日光温室无土栽培技术与后代初步鉴定等方面的关键技术,建立了枣树杂种规模化高效创制技术体系。通过体系应用实践,种仁获得率提高到11.90%,较人工杂交方式种仁获得率的0.01%效率显著提高,较常规田间播种可有效缩短育种周期11个月,取得了枣树人工控制杂交育种的突破性进展。3年控制杂交共获得果实6万余个,获得种仁7138个,成苗2089株,得种率11.90%,成苗率29.27%,构建了7个杂交代群体,为枣树遗传变异规律、重要性状QTL定位、基因图位克隆、分子辅助选择育种等研究工作奠定了基础。

关键词:枣;规模化杂交;杂种;技术

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Establishment and application of large-scale hybrid creation system in Chinese jujube

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Abstract: Hybridization is the most important way to create new varieties of fruit crops. However, there are some serious problems in cross breeding in terms to Chinese jujube, including difficulty in manual emasculation due to small flower, low fruit setting rate and serious embryo abortion. Only approximate 0.01% of pollinated flowers can produce fruits with hybrid seeds in conventional manual hybridization, which definitely hinders the progress of hybridization. It is significant to improve the breeding technology and promote the development of jujube industry by establishing a system of large-scale hybrid creation in Chinese jujube and breeding new artificial hybrid varieties to meet the urgent need for hybrid varieties in production. On the basis of large-scale hybrid creation system practice, a systematic summary is made for the key technical links in this article, including high-affinity hybrid combinations, bee-and-net-aided controlled cross, hybrid culture and identification with SSR, add-generation evaluation with soilless culture technology in greenhouse and hybrid preliminary evaluation. At last, a large-scale and high-efficiency controlled cross breeding system was established. In terms to selecting the parents, the combinations of 'JMS2'×wild jujube 'Xing16', 'JMS2'×'Jiao5', 'Dongzao'×'Chen-

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guang', 'Dongzao' × 'Wuhefeng' and 'Dongzao' × 'Fengmiguan' had better affinity, which realized the large-scale fruit and seed setting, and were suitable for large-scale hybrid breeding of jujube. The triploid hybrid offspring of jujube from 'Dongzao' × 'Chenguang' was obtained for the first time, which opened up a new way of sexual polyploid hybridization breeding. It was convenient and effective to use bees auxiliary pollination technique by covering net, which was to replace artificial pollination and greatly reduced the cost of hybrid. The 50 mesh fabric net and bigger net room were good for bee pollination. Generally, it covered an area of 20-70 m² by putting one box beehive including two combs that can meet the demand for pollination. It was effective to place newly hatched young bees and bee pupae in the hive of netting chamber during the first flowering period. The number of pollination by honeybees showed two obvious peak curves between 11:00—14:00 and 16:00—20:00 in a day, with 18:00 (equivalent to 16:00 in the inland) as the highest peak of pollination activity in Xinjiang. Several factors that may affect hybrid formation were investigated, and the controlled cross technology was optimized. The proper allocation ratios of female 'JMS2' to male parent were 1:1-3:1, while that of female 'Dongzao' to male parent were 1:1. Girdling may improve fruit setting and seed acquisition in 'Dongzao'. Sowing seed directly in matrix showed much simpler and higher seedling rate than test-tube culture, which was suitable for large scale hybrid culture. It was easy and convenient to put seeds in 4 °C cold refrigerator instead of stratification, to soak seeds until emergence instead of direct seeding seeds and to accelerate bud and use matrix seeding method, which was helpful to cultivate hybrid seeds. Hybrid seedlings emerged evenly and orderly, the average seedling rate of hybrid seedlings was about 50%, and at last a large number of seedlings were obtained. Male sterility 'JMS2' had the characteristics of self-fertility, and the obtained seeds were real hybrids. The offspring that used 'JMS2' as female parents in control cross breeding was real hybrids, which can keep their molecular identification. The greenhouse planting combined with the sand culture trough by soilless culture technology could realize that the 2 years old hybrid plants started flowering and fruiting and bore twice per year, which can be used for the preliminary identification of the traits of the offspring. Based on the study, a large-scale and high-efficiency controlled cross breeding system was established by integrating the utilization of male sterile germplasm, high-affinity hybrid combinations, bee-and-net-aided controlled cross, hybrid identification with SSR and add-generation evaluation in greenhouse. With this system, the breeding efficiency increased from 0.01% to 11.90% compared with that of conventional artificial pollination. The breeding cycle from controlled crossing to offspring preliminary identification was condensed into 30 months, which effectively shorted the breeding cycle about 11 months compared with the 41 months of the conventional breeding cycle. Using this system, more than 60 000 fruits, 7138 seeds and 2 089 seedlings were obtained in 3 years, with seed yield of 11.90% and seedling yield of 29.27%, and seven groups of hybrid population were obtained. The establishment of these hybrid populations provided materials for the genetic variation of male sterility, polyploidy, non-nuclear traits and fruit characteristics, and it also laid a material foundation for the research on QTL mapping, map-based cloning, and molecular assisted selective breeding for important traits in jujube trees. Therefore, a breakthrough in artificial control cross breeding of jujube was made.

Key words: Chinese jujube; Large-scale hybridization; Hybrids; Technology

杂交育种是果树培育新品种的重要手段之一，在众多果树的遗传改良中发挥了重要作用^[1-3]。枣

树是原产中国的特色果树，也是中国重要的干果之一^[4]。枣树由于具有花朵小、去雄难、坐果率极低和

胚败育严重等特点,常规人工授粉杂种得率通常只有万分之一左右^[5],因此,常规杂交育种十分困难,导致枣树的杂交育种进展相对滞后,至今未见人工杂交枣新品种的报道。长期以来枣树的育种一直采用实生选种和株系选优等方法,近几年,枣倍性育种和杂交育种开始取得进展^[6-7]。随着枣雄性不育品种的控制蜜蜂授粉杂交技术的应用,枣树免去雄杂交育种技术体系得以实践和完善^[5]。本文在课题组多年实践探索的基础上,系统归纳总结了枣树亲本的选配、蜜蜂辅助授粉、提高亲本结实率、杂交后代培养、杂种免分子鉴定及杂种日光温室加代培养等方面的关键技术和进展,建立了枣树杂种规模化高效创制技术体系,并将之应用于实践,实现了枣杂种的规模化创制,取得了枣树人工控制杂交育种的突破性进展。

1 亲本的选择与选配

亲本的选择选配是决定杂交育种成功与否的关键。一般杂交后代群体的多种经济性状大多表现趋中或退化趋势,从双亲性状的平均值可大体决定杂种的表现趋势,因此亲本的选择需要考虑具有目标性状的品质和较强的传递力,以及具有最多优点和最少缺点。亲本的选配主要侧重亲本间性状的优缺互补、主要性状的基因型和遗传特点^[8]。枣树有性杂交育种的关键障碍之一是优良品种的胚败育严重,大多数优良品种很难获得种仁^[9],因此,选择性状优良且含仁率高的母本是关键之一。另外,枣树自花结实能力较强,如果选择雄性不育品种或者花粉生活力弱的品种做母本,可以解决去雄难题,大大简化育种程序^[10]。枣树控制杂交的亲本选择选配方面,母本应选择雄性不育或自花不育,含仁率高且种仁饱满的品种,而父本选择花粉量大、花粉生活力强的品种,双亲间应具有较高的亲和性。前人研究认为‘冬枣’的鲜食品质极佳,花粉生活力几乎为零,且授粉后70%以上的胚能够正常发育,是理想的母本材料^[11],并通过杂交亲和性、结实率及创制杂交后代研究与实践,得出‘冬枣’×‘大叶无核枣’、‘冬枣’×‘苹果枣’、‘冬枣’×‘临猗梨枣’、‘冬枣’×‘映山红’、‘冬枣’×‘金丝小枣’、‘冬枣’×‘尖枣’、‘冬枣’×‘无核枣’、‘冬枣’×‘木枣’、‘冬枣’×‘小芽枣’和‘冬枣’×‘映山红’等杂交组合表现较佳^[12-18],部分组合成功获得杂交后代并构建了遗传连锁图谱。在控制杂交中

应用雄性不育种质,可有效解决去雄的难题,极大提高育种效率,是理想的母本材料。前人研究‘JMS1’‘JMS2’‘早脆王’均是雄性不育品种,设置的‘JMS2’×‘邢16’、‘JMS2’×J5、‘早脆王’×‘灵武长枣’、‘早脆王’×‘伏蜜桃’组合具有较高的亲和性,可获得杂交子代^[7,19-20]。

课题组前期研究发现‘冬枣’花药中虽含有花粉,但育性不高,通过观察发现其花粉粒椭圆形较多,萌发孔只有1个,而大多数枣品种的花粉粒为四面体形状、有3个萌发孔,推测这是造成‘冬枣’花粉萌发率极低的原因之一^[11,21]。闫超^[22]通过对2个国家资源圃不同品种的自花结实、自花可育性、自然授粉后的结实特性和育性等进行了系统研究,发现3个冬枣品系,都表现自花可实不育,自花结实率为25.00%~62.93%,自花可育率为0%。笔者在南疆引种发现,雄性不育种质‘JMS2’的不育性状表现稳定,而雄性不育种质‘JMS1’和‘冬枣’单花药花粉量少且花粉活力低,也具备稳定的雄性不育性状。同时,以上3个品种坐果率和得种率高,果实品质优良,是理想的母本材料。以‘蜂蜜罐’‘无核丰’‘交5’‘灰枣’‘中秋红’‘辰光(4X)’‘酸枣’‘邢16’等品种为父本,共设置了16个双亲及多亲杂交组合,采用免去雄控制蜜蜂授粉代替人工授粉经3年的育种实践,共获得7个杂交后代群体。分别以‘JMS2’‘JMS1’为母本构建的6个双亲杂交组合,3年共获果实15 097个,其中3 296个含有种仁(表1),以‘JMS2’的含仁率最高,经培养有1 079个杂种萌发出苗,成苗833株。以‘冬枣’为母本构建的3个双亲杂交组合,3年共得果实24 233个,得种仁650个(表2),经培养有397个萌发出苗,成苗364株。分别以‘JMS2’和‘冬枣’为母本配置了多亲本杂交大组合7个,2年共获得果实19 138个,种仁2 698个,成苗893株,构建了3个半胞后代群体。研究发现‘冬枣’与四倍体‘辰光’杂交,首次获得枣三倍体杂交后代^[23],开辟了多倍体有性杂交育种新途径。果树杂交育种的坐果率与亲和性指数密切相关,杂交亲本的结实率高低不仅反映了亲本之间的亲和性,还是判断组合优劣的重要指标^[24-25],因此认为,‘JMS2’与‘邢16’‘交5’‘灰枣’;‘冬枣’与‘辰光’‘无核丰’‘蜂蜜罐’均有较好的亲和性,实现了亲本的规模化结实和结籽,适用于枣树人工控制杂交育种规模化杂种创制。

表1 雄性不育为母本双亲杂交组合的结实及种子形成情况^[26]
Table 1 The fruit setting and seed formation of two-parent cross combinations with male sterility as female^[26]

杂交组合 Cross combination	2014年 In 2014			2015年 In 2015			2016年 In 2016			合计 Total					
	罩网数 No. of covering net	果实数 No. of fruits	种仁数 No. of seeds	含仁率 The seed rate/%	罩网数 No. of covering net	果实数 No. of fruits	种仁数 No. of seeds	含仁率 The seed rate/%	罩网数 No. of covering net	果实数 No. of fruits	种仁数 No. of seeds	含仁率 The seed rate/%	总果数 Total fruits	总仁数 Total seeds	平均含仁 率 Average seed rate/%
JMS2×邢16 JMS2×Xing16	1	329	23	6.34 a	2	841	517	30.15 b	2	2 303	1 690	72.97 a	3 473	2 230	36.49 a
JMS2×交5 JMS2×Jiao5	1	246	81	40.95 a	2	435	52	6.61 c	2	1 732	654	38.44 b	2 413	787	28.67 a
JMS2×无核丰 JMS2×Wuhufeng	1	174	1	0.35 a	1	122	0	0.00 c	1	623	1	0.16 d	919	2	0.17 a
JMS1×邢16 JMS1×Xing16	1	116	74	40.14 a	1	84	4	4.88 c	1	374	8	2.10 cd	574	86	15.71 a
JMS1×无核丰 JMS1×Wuhufeng	1	51	5	9.80 a	1	7	4	55.00 a	1	244	6	2.31 cd	302	15	22.37 a
邢16×JMS2 Xing16×JMS2	1	6	4	33.33 a	2	529	6	2.42 c	2	1 058	29	2.89 cd	1 593	39	12.88 a
邢16×JMS1 Xing16×JMS1	1	954	109	18.67 a	1	91	3	1.10 c	1	350	25	8.33 c	1 395	137	9.37 a
交5×JMS2 Jiao5×JMS2	1	370	0	0.00 a	2	334	0	0.00 c	2	1 346	0	0.00 d	2 026	0	0.00 a
无核丰×JMS2 Wuhufeng×JMS2	1	688	0	0.00 a	1	130	0	0.00 c	1	1 550	0	0.00 d	2 368	0	0.00 a
合计 Total	5	2 934	297	-	7	2 573	586	-	7	9 580	2 413	-	15 097	3 296	-

注:同列数据后不同小写字母表示在 $p < 0.05$ 水平上差异显著。下同。

Note: Different small letters indicate significant difference at $p < 0.05$. The same below.

表2 ‘冬枣’双亲杂交组合的结实及种子形成情况^[26]

Table 2 The fruit setting and seed formation of two-parent cross combinations with ‘Dongzao’ as female

杂交组合 Cross combination	2014年 In 2014			2015年 In 2015			2016年 In 2016			合计 Total					
	罩网数 No. of covering net	果实数 No. of fruits	种仁数 No. of seeds	罩网数 No. of covering net	果实数 No. of fruits	种仁数 No. of seeds	罩网数 No. of covering net	果实数 No. of fruits	种仁数 No. of seeds	罩网数 No. of covering net	果实数 No. of fruits	种仁数 No. of seeds	罩网数 No. of covering net	果实数 No. of fruits	种仁数 No. of seeds
冬枣×辰光 Dongzao×Chenguang	1	39	6	3	19	9	3	316	279	3	3 116	279	3	3 174	296
冬枣×无核丰 Dongzao×Wuhufeng	1	36	0	3	67	6	3	4 028	196	3	4 028	196	3	4 131	202
冬枣×峰蜜罐 Dongzao×Fengmiguang	0	-	-	1	45	20	1	1 596	82	1	1 596	82	1	1 641	102
辰光×冬枣 Chenguang×Dongzao	1	167	0	3	259	0	3	674	1	3	674	1	3	1 100	1
无核丰×冬枣 Wuhufeng×Dongzao	1	187	0	3	1 113	5	3	9 648	16	3	9 648	16	3	10 948	21
峰蜜罐×冬枣 Fengmiguang×Dongzao	0	-	-	1	668	10	1	2 571	20	1	2 571	20	1	3 239	30
合计 Total	2	429	6	7	2 171	50	7	21 633	594	7	21 633	594	7	24 233	650

2 蜜蜂辅助授粉技术

利用罩网控制蜜蜂辅助授粉技术是替代人工授粉的简便易行且有效的方法,也大大降低了杂交成本^[27]。枣树具备花小、花量大、花期长的特点,高效的蜜蜂辅助授粉技术是规模化创制杂种的前提,也是枣树人工控制杂交育种的关键技术环节。罩网控制蜜蜂授粉首先要解决网的问题,一般采用尼龙纱网为罩网材料^[20]。笔者研究发现尼龙网眼密,利用尼龙纱网罩网放蜂,不利于通风透光,网室内亲本坐果率低,而且尼龙网成本高、缝制工序繁琐,不适宜大规模杂交试验的应用。进一步实践筛选最终发现轻薄透气的白色蚊帐纱网布通风透光效果最好,网室内的光照、温度和湿度与外界差异不大,且成本低,宽幅大易于缝制,是大规模开展控制杂交试验的理想材料。

有效的蜜蜂数量配置是影响种子产量和质量的影响因素^[28],笔者研究中发现枣树体大,面积为20~70 m²网室配置1箱2脾蜂已经可以满足授粉需要。同时发现,在初花期网室蜂箱内放置刚孵化的幼蜂及蜂蛹效果好,幼蜂出巢后即可开展授粉工作,可直接适应杂交小网室的环境空间,也有效避免了外来花粉的污染;而直接放置老蜂,由于活动范围受限,直接撞网死亡的蜜蜂数量偏多。另外,杂交期间需要每星期放置新鲜的糖水和清水饲养蜜蜂,也有报道蜜蜂在不提供食物饲喂时,其访花频率和单花停留时间均显著高于提供食物。前人观察发现蜜蜂上午比下午活动频繁,以10:00—11:00、12:00—14:00效率最高^[29-30]。南疆阿拉尔垦区夏季中午光照强,枣树杂交罩网时蜜蜂授粉活动时间一般持续在11:00—14:00,16:00—20:00两个时间段,其中18:00达最活动高峰,而且蜜蜂活动高峰期与枣花的绽放期相吻合,此时正处于枣花粉活力高,柱头可授性强时期^[31]。一般网室过小会限制蜜蜂的活动空间,本研究依据树体的大小和行间距设置网室的宽度为3.5 m,长度分别为4、6、8、20 m,高度2.5~3 m,可满足蜜蜂的授粉活动。观察发现20 m长的大网室,由于蜜蜂的活动空间较大,蜜蜂撞网的数量相对较少;4 m的小网室,放蜂初期会出现大量网室四角部集聚,撞网死亡数量较多^[26]。因此,南疆枣树罩网控制蜜蜂辅助授粉,最好选用50目白色蚊帐纱网布,根据亲本株数设置大网室,一般在5

月初开花前罩网,初花期放蜂,每网室放置1箱2脾蜜蜂,以刚孵化的幼蜂为主,每周定期更换糖水和清水。

3 提高亲本结实率技术措施

优化管理技术提高亲本结实率和含仁率是获得规模化杂种的保障。研究发现不同年份的气候条件以及不同的栽培管理措施对‘冬枣’坐果影响较大,一般大树坐果多、小树坐果少,合理环剥改善树体营养是提高‘冬枣’坐果率的重要因素^[32]。经实践发现,在南疆以灰枣为砧木高接亲本,当年可少量坐果结实,一般嫁接3年后树体可实现大量结果,因此枣树杂交育种应尽量选择树龄超过3a的大树。另外,环境条件对‘冬枣’结实率影响较大,在温度26~28℃,空气相对湿度在75%~95%,光照充足条件下适宜授粉坐果。

另外,亲本配置比例是影响结实和结籽的因素之一。经多年杂交实践发现,雄性不育为母本的双亲组合中母本与父本的单株配置比例适宜在1:1~3:1之间,母本株数大于父本株数有利于提高结实率。‘冬枣’×‘辰光’组合中母本与父本单株配置比例适宜在4:3~1:1之间,‘冬枣’×‘无核丰’组合适宜比例为3:2~1:1,杂交中可根据树体大小稍作调整^[26]。不同亲本间最好交替或混合嫁接,有利于提高蜜蜂传粉效率和提高杂交果实含仁率。

合理修剪与负载量,是影响杂交结果数和含仁率的重要因素。一般根据树体大小每单株留新生枣头1~3个,每枣头留二次枝2~3个,每二次枝留枣吊5~6个,定期疏除新生枣头。花期每周喷施0.3%的磷酸二氢钾和0.2%硼酸,6月中旬进行基部环割,‘冬枣’大树根据树体大小可适期环剥。坐果后及时穴施复合肥,增加树体营养,定期防治病虫害,杂交过程中每个环节都需要进行精准的树体管理。

4 杂交后代的培养

果树杂交育种上多采用胚培养技术对幼胚进行胚挽救,有效克服了胚的早期败育并获得种质资源^[33-34]。枣树杂交育种也开展了胚培养的研究^[35-36],但均停留在方法研究上,未实现规模化胚培养。笔者对冷藏处理2个月后的成熟胚进行胚培养播种,杂交后代的平均成苗率为34.15%,发现多数子叶正

处于萌动状态,只能通过诱导愈伤组织途径成苗,育种周期长且成苗率低。由于枣种子外包被着坚硬的种核,组培条件下剥取种仁难度大,极容易感染细菌,严重影响了子代的成苗率。另外,枣组培苗移栽后的2~4周幼苗不适应外界环境容易死亡,致使最终仅有14.26%的幼苗在温室移栽成活,因此认为,组培培养成苗效率较低,驯化移栽技术还需完善。

经多年实践探索出简便易行的种仁贮藏及基质播种培养方法。一般于9月中旬至10月中旬采收果实,去除果肉并将枣核自然晾干,放入透气的尼龙网兜,置于4℃冰箱内冷藏处理2个月,利用冰箱4℃冷藏处理代替层积处理。期间定期翻看种核,可包裹报纸防止种核受潮。12月底至1月初取出种核,剥出种仁,45℃温水浸种至室温放置24h,置于光照培养箱28℃催芽处理,待1/3种子露白后即可播种。准备32孔穴盘,混合基质配方为 $V_{草炭}:V_{珍珠岩}:V_{蛭石}=3:1:1$,60%含水量,温室内播种并覆膜,7~10d出苗,15d时调查出苗率,喷施1/2完全营养液促进生长,待真叶4~6枚时可移栽。采用浸泡催芽方法较种子直接播种、沙藏翌年播种的杂种苗出苗均匀整齐,出苗量大,是有效获得杂种后代的播种方法,最后用穴盘基质播种育苗代替田间土壤育苗。实践证明采用基质穴盘播种育苗方式,2015—2016年获得的杂交种子,采用播种的方式培养,其中双亲组合共播种2606个种仁(表3),出苗1331株,成苗1181株,平均出苗率在50%左右,平均成苗率约45%;多亲组合共播种2291个种仁,出苗948株,成苗893株,出苗和成苗率分别为41.4%和39%。通过控制杂交与基质播种培养,共获得温室小苗2074株,具有明显成效。可见穴盘播种方法具有操作简单、高效易行、且可规模化实施的特点,可用于大批量杂种的培养。

5 杂交后代鉴定

一般杂交后代可采用SSR标记等鉴定,其准确性高,技术相对成熟^[18,37]。张钟鉴定‘冬枣’×‘梨枣’的杂交后代57个,未发现‘冬枣’自交后代^[38]。郭敬丽^[6]通过控制杂交途径获得13个冬枣和无核枣后代,经RAPD标记鉴定到了12个冬枣×无核枣杂种,1个无核枣×冬枣杂种。笔者利用SSR标记鉴定获得的‘冬枣’×‘辰光’杂种子代群体。枣树自花

表3 双亲杂交组合的播种成苗率^[26]

Table 3 The seedling rate of two-parents crosses after sowing

杂交组合 Cross combination	2016年 In 2016					2017年 In 2017				
	播种量 No.of seeds	出苗量 No.of seedlings	出苗率 Rate of seedlings/%	成苗量 No.of plantlets	成苗率 Rate of plantlets/%	播种量 No.of seeds	出苗量 No.of seedlings	出苗率 Rate of seedlings/%	成苗量 No.of plantlets	成苗率 Rate of plantlets/%
JMS2×邢16 JMS2×Xing16	452	274	60.62	181	40.04	1 004	435	43.33	421	41.93
JMS2×交5 JMS2×Jiao5	47	34	72.34	24	51.06	480	197	41.04	197	41.04
冬枣×辰光 Dongzao×Chenguang	9	5	55.56	4	44.44	279	206	73.84	192	68.82
冬枣×无核丰 Dongzao×Wuhefeng	4	0	0.00	0	0.00	208	126	60.58	111	53.37
冬枣×蜂蜜罐 Dongzao×Fengmiguan	20	8	40.00	8	40.00	76	46	60.53	43	56.58
合计 Total	532	321	60.32	217	40.79	2 074	1 010	48.70	964	46.48

结实不育是枣树的基本类型^[39],闫超^[22]通过对国家资源圃不同品种的自花结实、自花可育性、自然授粉后的结实特性和育性的系统研究,发现冬枣自花可实不育,其自花结实率为25.00%~62.93%,自花可育率为0%。可见,控制杂交过程中‘冬枣’为母本所得后代可保证杂种的真实性。笔者曾在连续5年的控制杂交过程中,将雄性不育(‘JMS2’‘JMS1’)和‘冬枣’等品种设置罩网不放蜂处理,与罩网放蜂处理进行对比,发现罩网不放蜂条件下雌性不育JMS2和冬枣可以自花结实,但连续3年均未获得种仁,因此,雄性不育品种具备自花结实不育特性^[26],应用雄性不育品种为母本的双亲控制杂交组合中,只有一个花粉来源,雄性不育母本所获种子是真实杂交种,在控制杂交育种中可免于分子鉴定^[40]。

随着控制杂交育种技术的完善,大规模杂交育种得以实现。规模化控制杂交一次可获得成千上万个后代,若采用分子鉴定不仅时间漫长,而且成本昂贵,寻找一种免于分子鉴定的杂交方法意义重大。应用雄性不育‘JMS2’‘JMS1’和‘冬枣’为母本的双亲控制杂交组合,在保证隔离精准条件下所获的后代应为真实杂种,可免于分子鉴定,雄性不育种质在罩网控制杂交育种中的应用,提高了育种效率,保证了杂交的高效和真实性,在枣树规模化杂交育种上具有重要的应用价值。但是,在其他优良品种组合杂交中,子代仍需进行杂种的分子鉴定。

对杂交后代的表型性状调查进一步鉴定杂种的真实性。以‘JMS2’为母本的2个杂交后代叶片和果实性状均发生不同幅度变异,其中叶片和果实

大小的F1均值明显比亲中值低,出现趋小变异趋势,果实形态部分与亲本相似,大部分果实形态发生了扁圆形、长圆形及倒卵圆形的变异。‘冬枣’×‘辰光’的后代部分出现了多倍体的巨大性状,其叶片形态、叶片颜色、叶片厚度及叶片状态与父本‘辰光’表现一致;叶面积介于父母本之间,叶片锯齿宽度与父本性状接近,叶片气孔大小及气孔密度与父本‘辰光’表现一致;部分后代单株果实形态出现了扁圆形变异。通过表型鉴定,认为控制杂交育种体系所得后代为真实杂交后代。

6 日光温室无土栽培技术与后代初步鉴定

日光温室促成栽培可实现枣果实提前40~60 d上市,在枣树鲜食枣生产中广泛应用。将枣杂交亲本定植在日光温室内,通过控制温室环境实现亲本的提早坐果,可实现提前2个月收获杂交果实,可有效缩短了育种周期。2月份将基质播种培养的后代小苗在日光温室条件下定植,选用现代化无土栽培技术。沙培槽定植,沙子、草炭混合基质栽培,安装滴灌带每周滴灌1~2次完全营养液。无土栽培技术培养杂交后代,移栽成活率高,易于管理,且养分供给充足。一般定植当年5月份可揭开温室薄膜生长,翌年1月扣棚升温,2月份萌芽,3月份抽生枣头,水平拉枝45℃处理,4月初每枣头留3~4个二次枝摘心处理,促进4月下旬至5月下旬第一批开花,开花期间滴灌高磷钾营养液。6月初坐果后疏除单株下部未坐果二次枝及枣吊,促进新生枣头萌发,滴灌完全营养液,揭开棚膜接受正常光

照照射,7月中旬至8月下旬第二批次花开,开花期间滴灌高磷钾营养液。开花期每10天喷施1次芸苔素·赤霉素,促进杂交后代坐果和果实膨大。8月下旬第一批果实采收,初步鉴定后代性状,10月下旬至11月上旬第二批果实采收,进一步鉴定后代性状,筛选优良单株,完成初选。12月份可采集实生后代接穗,高接进一步复选鉴定。此模式可实现二年生子代单株当年2次开花。因此认为,日光温室无土栽培技术不仅可以实现杂交后代的加代培养,还可作为杂种资源圃进行优异种质的初步选优。笔者实践发现成苗当年可根据叶片大小、树体长势等性状初步筛选优异单株,第二年即可有50%以上单株开花结实,可直接进行果实的初步评价筛选,第三年80%以上单株可开花结实,进入果实评价筛选时期,对筛选出的优异后代单株可在田间高接进一步鉴定评价果实的性状。

7 技术体系的应用实践

将常规人工杂交育种与罩网控制杂交育种的用工、结实率及育种效率进行比较。人工杂交中人均每天只能去雄和授粉约160朵,每年5~7人、7~10d,仅能授粉约8000朵,能获得果实40个左右、种子10个左右,坐果率只有约0.5%,杂种得率只有0.01%^[8],杂交3年仅得几十个后代,人工去雄杂交效率极低。而采用控制杂交育种,第一年扣网9个,第二、第三年分别扣网18个,扣网面积0.13~0.2hm²。从5月中旬扣网到7月中的管理只需3~4人,每人实际工作10d左右,3年共得到果实6万个,得到种仁7138个,成苗2089株,坐果率在1%~10%,得种率11.90%,成苗率29.27%。通过控制罩网杂交,杂交效率提高1000多倍,首次获得规模化杂种后代群体。

将控制杂交和常规杂交的育种周期进行比较。常规杂交选择第一年的6—7月份授粉,10月份采收果实,层积处理后翌年春天5月份播种,生长期包括翌年的5—10月,第三年5—10月,第四年6月多数单株进入开花期,第四年9月份结果后初步进行性状评价,共计从亲本杂交到后代鉴定和初选需跨时四年共计41个月。而控制杂交同样选择在第一年的6—7月份授粉,10月份采收果实。之后4℃冷藏2个月代替层积处理,12月份播种育苗,温室定植生长,生长期包括翌年的1—11月,第

三年的2—3月,营养生长达13个月,第三年的4月多数单株进入开花期,8月份结果可进行果实的初步评价,共计从亲本杂交到后代鉴定和初选需跨时三年共计30个月。体系的运用较常规田间播种至少提前1~2年,有效缩短了育种周期。

2014—2017年应用枣树杂种规模化高效创制技术体系进行了多个亲本的控制杂交,首次构建了4个双亲杂交组合的后代群体,其中‘JMS2’×‘邢16’获得杂交后代株系602个,‘JMS2’×‘交5’获得杂交后代株系221个,‘冬枣’×‘辰光’得到后代株系197个,‘冬枣’×‘无核丰’获得到后代株系111个,同时获得‘冬枣’的半胞杂交后代株系531个,‘JMS2’的半胞杂交后代株系124个,‘中秋红’的半胞杂交后代株系121个,初步构建了3个半胞杂交后代群体^[26]。

8 结 论

基于枣杂种创制实践,建立了融雄性不育种质利用、高亲和力杂交组合选配、罩网隔离蜜蜂辅助控制杂交、杂种SSR分子鉴定、温室加代评价等于一体的枣树规模化高效杂交育种技术体系。通过体系应用实践,种仁获得率提高到11.90%,较人工杂交方式种仁获得率的0.01%效率显著提高,较常规田间播种可有效缩短了育种周期11个月,取得了枣树人工控制杂交育种的突破性进展。应用枣树杂种规模化高效创制技术体系首次构建了4个双亲杂交组合的后代群体和3个半胞杂交后代群体,3年控制杂交共获得果实6万余个,获得种仁7138个,成苗2089株,得种率11.90%,成苗率29.27%。这些杂交后代群体的构建为今后枣树雄性不育、无核和果实性状等重要性状遗传变异规律研究、重要性状QTL定位、基因图位克隆及分子辅助选择育种等奠定了基础。

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