Genetic insights on single cross maize hybrid and its importance on maize self-sufficiency in Nepal

Pabitra Joshi¹ and Damodar Gautam²*

¹Agriculture and Forestry University (AFU), Rampur, Chitwan, NEPAL
²National Maize Research Program (NMRP), Rampur, Chitwan, NEPAL

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INTRODUCTION

Maize (Zea mays L.) is the second most important crop after rice in terms of area coverage, production and productivity (KC et al., 2015), providing nearly 20% of total cereal production and 30% of the calories in Nepal. Maize consumption per capita was 98 g/person/day in Nepal, the highest in South Asia. Demand for maize has increased by 5 percent over the last decade. MOAD/CBS (2016) recorded an 11 percent decrease in the maize import dependence ratio over the period 2008-2014. The growing trends in the consumption of more protein and micro-nutrient rich food products in Nepal have been observed in recent days compared with the last decades, says MoALD (2017). Maize being one of the major food crops of Nepal, from the view point of food consumption by human and animals, there is less gap between total demand and national production, but from the feed industry side, demand gap is drastically higher (Dhakal et al., 2015). The demand in Nepal is shifting from food to livestock feed and poultry, according to KC et al. (2015). It is estimated that the 114 feed industries produced about 0.5 million tons of feed per annum in Nepal.

We import about 80 percent of the needed maize with a corresponding annual trade deficit of around 12 billion rupees (USD 109.18 million) in fiscal year 2017/18 alone. Low yielding Open-Pollinated Varieties (OPVs) continue to grow a wide segment of small farmers in the marginal areas. In Nepal, the average national maize yield of 2.67 t ha⁻¹ is often extremely small, which is around 50% of the global average. However, a big yield of food consumption by human and animals, there is less gap between total demand and national production, but from the feed industry side, demand gap is drastically higher (Dhakal et al., 2015). The demand in Nepal is shifting from food to livestock feed and poultry, according to KC et al. (2015). It is estimated that the 114 feed industries produced about 0.5 million tons of feed per annum in Nepal.
difference exists between the real and potential yield of open pollinated varieties (OPV) and hybrids for both Nepal's hills and Terai. If we are able to increase maize production by 50 percent, the average national yield would hit almost 4 t ha\(^{-1}\). The emphasis should be on narrowing the yield gaps by increasing the farmers' access to better seeds and crop management practices. Hybrid maize seed is formally or informally imported from India every year through the porous border. NARC has produced 30 different maize varieties until 2016 (NMRP, 2013, 2015; NARC, 2016). In addition, Nepal has registered 34 imported maize hybrids (NMRP, 2013; NARC, 2015). The suggested hybrids aren't adequate to meet farmers' standards, according to NARC. The future is not so bright, even if formally registered hybrid is grown, some hybrids can't be as good as anticipated. In Nepal maize productivity is still very low among the Asian countries and therefore needs to be increased. The increased productivity will come from cultivating high yielding hybrids. Information on open pollinated varieties of Nepalese Heterotic Groups is not yet available. One of the reasons may be due to the limited knowledge provided about the Nepalese maize germplasm, genetic diversity and heterotic groupings (Melchinger et al., 1992). Approximately 45 million hectares of maize are grown in lowland tropics. Climate, biotic and abiotic constraints affect productivity severely. Adoption of suitable high yield hybrids not only increases grain yield and efficiency, but also contributes to higher incomes per hectare. Past experience shows that, Hybrid maize had covered around seven to ten percent area of Nepal in 2010 (Gurung et al., 2011; Thapa, 2013). Nepal imports nearly 100 percent of corn seeds every year (Adhikari, 2014) and nearly 100 percent of hybrid seed is being imported from India (Gurung et al., 2011). Recently, many research organizations are working to develop yield competitive and climate resilient hybrid maize varieties but result is not satisfactory. In fact, the grain yield performance of released cultivar is low as compared to multinational commercial hybrids and also seed availability and distribution of those hybrids is very poor for general cultivation. Scenario of unavailability of competitive Nepalese hybrid cultivars and underdeveloped seed industries caused dependency over imported hybrid maize seed every year (Gurung et al., 2011; Liao et al., 2011).

Most farmers in the Terai region of Nepal grow open pollinated varieties (OPVs). there is a potential to double the current national average maize yield by exploiting Heterosis. Nepal's Terai and inner Terai are highly potential for hybrid cultivation. Several attempts have been made for the hybrid maize production and distribution possibilities in Nepal (Kunwar and Shrestha, 2014, 2017). Farmers demand F1 seeds and a variety of multinational hybrids are marketed annually.

Global maize status
Maize is the most versatile, global food crop. When the world population rises, total crop production worldwide is not meeting increasing food demand. Global cereal demand is estimated to increase by more than 1000 million tons (56%) over the period 2000–2050. Since the land is small, we need to find alternatives that can produce higher yields in the given cultivable area. This obviously demonstrates the need for high yielding maize hybrids, writes Dass (2009). The focus on developing high yielding single cross hybrids that are resource-efficient under various soil and climatic conditions has led to many single cross hybrids being created. The main advantages of hybrids relative to population varieties are increased yields due to optimum use of Heterosis. Approximately 45 million hectares of maize are grown in lowland tropics, where a variety of climatic, biotic and abiotic constraints affect productivity severely (Pingali and Pandey, 2001). Significant progress has been made worldwide with regard to maize improvement using conventional breeding techniques and cultivating various kinds of high yielding hybrid varieties. The problems are complex and varied and there is no single technical solution to them (Figure 1).

History of hybrid maize
G.H Shull and E.M East laid the theoretical foundations for the system of inbred-hybrid breeding. East and others originally didn’t believe this approach could be commercially successful. Jones (1918) invented the double cross model. A double cross is formed by creating two single cross hybrids (A/B) and (C/D) and then crossing the two single crosses the next season and selling the seed from the second cross to growers. Inbred and hybrid growth processes culminated in incrustations with efficiency per se improved. The basic genetic cause of Heterosis has been discussed since Shull's initial papers (Shull, 1908, 1909). This is defined under Heterosis, heading. The definition of single crosses which includes only two parents A x B was introduced in the 1970s.

Heterosis
Heterosis brings increases in grain production, earlier flowering, a higher number of leaves, taller trees, more tillers and panicles per plant, heavier seeds, more seeds per panicle (Acquaah, 2012), a quicker rate of emergence of seedlings, more vigorous seedlings and a higher dry weight of plumes (Cisneros-López et al., 2007; León-Velasco et al., 2009). The Heterosis hypothesis has been widely exploited in crop breeding, resulting in a large increase in yield. This phenomenon is identified with Heterosis when the parents are taken from different populations of the same species (Charlesworth and Willis, 2009). The three key theories came to describe; Jones (1918) Heterosis phenomenon through dominance, Hull, (1945) through Over-dominance, and Powers (1944) through epistasis. Heterosis applies the dominance theory to the accumulation of beneficial dominant genes or the masking of deleterious recessive alleles in the hybrid (Davenport, 1908; Bruce, 1910; Keeble and Pellew, 1911). Heterosis occurs in quantitative genetic terms when there is a degree of directional superiority (d), and the parents vary in the frequency of genes (Bruce, 1910; Falconer, 2003).

The hypothesis of dominance can be formulated in terms of a single locus (B) with no epistasis as

\[
\text{Heterosis} = d - \frac{1}{2}\text{a} + \frac{1}{2}\text{a} = d - \frac{1}{2}\text{a}
\]
Where, $a$ and $-a$ are the genotypic values of the parental genotypes (B1B1 and B2B2) and $d$ is the genotypic value of the non-parental genotype (B1B2).

The dominance hypothesis is consistent with recent genomic evidence of differences in gene content between maize inbred lines. The other theory, over-dominance, suggests that the heterozygous combination of the alleles at a single locus is superior to all of the homozygous combinations. Unlike the dominance theory, it also allows the parents to vary in the abundance of genes (Russell, 1986). The parents must be genetically diverse in order for Heterosis to occur and thus the allele frequencies of successful Heterotic groups must vary (Smith et al., 1990). It is clear that well-developed Heterotic patterns of mature breeding programs are artificial buildings created by breeders, and improved by the breeding hybrid process (Tracy and Chandler, 2008). The basis for modern Heterotic group was established by maize breeders in the 1940s simply by dividing the available inbreds into two classes in a seemingly arbitrary manner with little regard to phylogeny. Cress (1967) proposed that the way to make the most of a mutual recurrent selection system is to create one pool with the available germplasm and then arbitrarily divide the pool into classes. When developing hybrid maize it involves inbred development, determining the best inbred combination, evaluating the hybrids for superior hybrid selection. Conducting multi-location trials for two to three years and their evaluation are the most critical measures for launching hybrid maize cultivars for final release. There is a revival of vigor in hybrids that has been lost through inbreeding and dominance in the expressed trait(s) leading to various advantages such as high-quality food, disease tolerance, insect and pest, and some more agronomic traits. The crop has achieved genetic advantage in yield and resistance and tolerance to biotic and abiotic stresses after several decades of breeding research with the use of diverse germplasm.

**Combining ability**

Combining ability is an important feature of hybrid breeding. Combining capacity analysis is of particular importance in cross pollinated crops such as maize (Sharma et al., 2004). It helps to identify potential parents that can be used to produce hybrids and synthetics. The idea of combining capacity has become increasingly relevant over the years not only in maize. A sound breeding system offers the possibility of producing high-yielding crop varieties, says Griffing (1956). The diallel crosses are widely used in plant breeding programs to measure the GCA and SCA effects of agronomic traits. The top-cross test which involves crosses of corn lines with a tester is another method for estimating GCA. The study is more effective because it provides valuable knowledge about hybrid success in selecting parents. It also supports selection efficiency in isolated populations, according to Bočanski et al. (2009) and specific combination ability provides the performance of any two inbred inbred lines in a hybrid combination. It is useful for selecting the maize hybrid parental lines. It helps to get an idea for a specific character about the nature of the gene action. Diallel cross analysis offers estimates of genetic parameters for combining ability and a description of the parents’ dominance relationship examined with or without reciprocals using the first filial generation (F1).

**Maize heterotic group**

In any breeding program, Heterotic groupings are vital. Choosing Heterotic groups is crucial because heterotic patterns are valuable tools to manipulate the trait of interest to Heterosis. Within a Heterotic group, crosses are made to establish superior inbred lines within the group. Superior inbred lines are crossed from dissimilar heterotic lines to create hybrids. Each year, several hundred’s or 1000’s of hybrids are evaluated to determine the superior hybrids for commercial release based on their performance and target area of adaptation (maturity, stress, climate, etc.). The ears are harvested from female rows, and they are the hybrid crop. Hybrid seed is also formed by growing female and male rows and detasselling (removing male inflorescence from female row of plants). To avoid selfing amongst female inbred line, manual or mechanical tools are used to detassel before pollen shed. Heterotic groups suggest that plants derive from related populations or from different groups that exhibit similar combination capacity and heterotic response when crossed. Globally, heterotic groups differ according to region or particular breeding program. The following table shows some examples of heterotic maize group around different countries around the world (Table 1).

**Major steps involved in hybrid maize development**

The major steps toward hybrid maize production begin with the creation of inbred lines. Inbreeding is a pairing of ancestor-related individuals more closely than would be expected under random mating. The most important step in a breeding program is to recognize parental combinations which can produce hybrids of superior yield. Extensive testing is important to select sources of germplasm with high mean efficiency, useful genetic diversity and excellent combination potential. Most of the efforts on population enhancing breeding programs involved population enhancement per se but restricted elite population hybrid research. The ‘population-hybrid concept’ is an alternative that exploits Heterosis using genetically broad-based Germplasm. It has been documented that parents with high general combining ability and large genetic distance generate hybrids with better yield output (Cox and Murphy, 1990; Diers et al., 1996). Extensive population hybrid testing should increase the use and exploitation of genetic diversity. Maize continues to rise rapidly due to a good combination of high market demands with comparatively low production costs and high yields. Inbred lines or hybrids are more promising source materials than populations with no inbreeding history. The CIMMYT germplasm has been one of the strongest examples of genetic diversification worldwide in the past decade. The introduction of exotic germplasm maize is an effective way of extending the genetic base of local maize germplasm (Nelson and Goodman, 2008).
The germplasm introduced from CIMMYT was a great resource for tropical germplasm improvement (Yuan et al., 2002; Xia et al., 2005). Superior hybrids are then tested and established for commercial seed production and used by farmers.

**Conventional Maize Hybrids (Involves inbred parents)**

Single Cross (A x B), Three-Way Cross (A x B) x C, Double-Cross (A x B) x (C x D).

**Non-Conventional-Low Cost Hybrids**

Top-Cross (Variety x Inbred), Double Top-Cross (Single Cross x Variety), Variety cross (Variety A x Variety B), Synthetic cross (Synthetic A x Synthetic B), Population cross (Population A x Population B) (Figure 2).

**Types of hybrids**

**Single cross hybrid**: Single cross hybrid is formed from crossing between male and female inbred line and have the lowest seed yield and whose seed price is also high. Such hybrids are highly standardized among all other hybrids and will deliver the highest grain yield when grown. In China, India, South Africa, Thailand, the USA, Vietnam, and other established seed markets, single-cross hybrids are most common. This is because farmers in these settings strive at higher yields and can afford to pay the higher price, provided that the seed cost is a lower percentage of overall costs than in low yield circumstances. In many cross-pollinated crops such as maize, single cross hybrids (F1) are commercially marketed as the first generation of selection (Miranda et al., 2008).

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**Table 1.** Showing some examples of Heterotic group and Heterotic pattern in Maize available around different countries in the world.

| Heterotic group | Heterotic pattern | Country | Reference |
|-----------------|-------------------|---------|-----------|
| U.S. dent lines, European flint lines | U.S. dent lines X European flint lines | Europe | Schnell et al. (1992) |
| Female group Stiff Stalk (SS) and the male group is designated Non-Stiff Stalk (NSS) | Stiff Stalk (SS) X Non-Stiff Stalk (NSS) | U.S Corn belt and Canada | Duvick et al. (2004) |
| Tang sipingtou and Luda honggu germplasm, Lancaster Sure Crop (LSC), Reid Yellow dent (RYD) | Germplasm, domestic X LSC, domestic X PN, Dom X Lan or Dom X Reid, Luda Red Cob X Lan | USA, China | Li et al. (2002, 2004) |
| Suawan, Reid, Non Reid | Suawan X Reid, Suwan X Non Reid, Reid X Non Reid | China | Fan et al. (2008) |
| Tuxpeno combines well with Cuban Flint, Coastal Tropical Flint (Caribbean Flint), Tuson, ETO, Perla and Chandelle | Tuxpeno combines well with Cuban Flint, Coastal Tropical Flint (Caribbean Flint), Tuson, ETO Cuban Flint combines well with Tuxpeno, Tuson, Coastal Tropical Flint, and Perla. Coastal Tropical Flint combines well with Tuxpeno, Cuban Flint, and Chandelle | China | Wellhausen (1978), Goodman (1985, 2005), Vasal et al. (1999) |

Source: (Meena et al., 2017).

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**Figure 1.** Ten years data for production, productivity and cultivated area of Maize globally (FAOSTAT).
Advantage of SCH over other hybrids

There are also benefits of manufacturing single cross hybrid. As in the production of SCH two parental lines are required so the production and maintenance are easier than three way cross or double cross which requires production and maintenance of three and four parental lines respectively. Expression of Heterosis is maximum in SCH and they are highly sensitive in high yielding and high input environment. As compared to three way and double cross hybrid they have uniform plant height, ear height, tasseling, silking, pollen shedding and all other characteristics. Because of the availability of excellent inbred female parents, single-cross hybrids constitute almost 90 percent of hybrid corn seed market. Nonetheless, SCH have few drawbacks as well including, the female parental line is typically low yielding resulting in high cost of seed production and the price of seed. Similarly, if any part is susceptible, it will affect all of the single-cross plants, resulting in lower results. Pollen shed occurs during a shorter period since all the plants are genetically alike, with the potential for lower yields, especially under stress condition (Beyene, 2016).

Modified single cross maize hybrid and its importance

One of the key problems facing breeders who grow maize hybrids is the inbreeding depression typically reflected in the inbred lines used as parents. Modified single cross hybrids provide a potentially desirable option to lower the costs of hybrid seed development. In this approach as an alternative to using an inbred line as the female parent, sister lines (SLs) are used in seed corn production (Rojas and Sprague, 1952; Wych, 1988). The hybrids developed using SLs are referred to as modified single-Cross (MSC) hybrids. One of the main reasons for using modified single crosses in maize is to increase the amount of seed produced. The yield of sister crosses was significantly higher than their parental inbreds and proposed that use of modified single crosses could significantly increase hybrid seed production. Liu et al. (1994) concluded that early generation sister crosses were stronger than later generation sister crosses. Lou et al. (1993) found that the yield of Sister Crosses was significantly higher. Also, Cordava et al. (2000) checked crosses of tropical sister lines separated at S3 inbred generation and registered yields close to those crosses using parental elite lines. They concluded that this breeding technique is useful to increase yield and reduce the cost of maize hybrid seed in developing countries. Seed industry reports from Mexico, Thailand, Vietnam, and Brazil indicate a tendency to shift from three-way cross hybrid to modify single and single cross hybrids. International Maize and Wheat Improvement Center (CIMMYT) have also conducted maize research with the main objective to generate awareness about the implementation of the modified single-cross hybrids model. Most of the MSC hybrids were not substantially different from the “best” SC counterpart. 11 out of 180 had grain yields that were slightly lower than their SC hybrid equivalents. Three of 180 hybrids had yields significantly greater than both SC hybrid counterparts. The SLs used in this study exhibited an average grain yield of seed. Similarly, if any part is susceptible, it will affect all of the single-cross plants, resulting in lower results. Pollen shed occurs during a shorter period since all the plants are genetically alike, with the potential for lower yields, especially under stress condition (Beyene, 2016).

Single cross hybrid seed production

The production of open pollinated maize seed is fairly straightforward, but the production of hybrid seed requires additional field practices. Development of hybrid maize seed requires intentionally crossing a female parent population into isolated fields with a male parent. Each hybrid variety consists of a...
specific combination of the parents being female (seed bearing) and male (pollen providing). The two parents’ field management is also important and requires careful planting timing, elimination of off types, and removal of tassels and separate harvesting of the female seed.

Biotechnological approaches in single cross maize hybrid development

Advance technologies have to be applied to increase the yield of single cross hybrids. This highlights the need for biotechnological approaches to advance the maize breeding strategies. Modern application of biotechnology in developing maize can be divided into two main categories: molecular genetics and genetic engineering. Functional genomics is intended to explain the role of all genes in an organism, and it will become an important biotechnology tool (Prasanna, 2008). The various steps involved in hybrid breeding programs such as multiple crosses and screening for superior performance and Heterosis combinations are very expensive, laborious and time consuming. If Heterosis can be predicted before the crosses are made, then the number of crosses to be performed and the progeny to be screened can be reduced considerably. Genetic diversity between the parents has been suggested as a potential predictor of Heterosis. The relationship between RFLP-based genetic distance and Heterosis depends on the form of crosses studied. In determining the correct breeding technique, the type of gene actions involved in hybrid success and their relative contribution are of particular importance. One theory for the Heterosis interpretation is over-dominance. The correlation coefficient for grain yield and genetic distances based on SSR markers between mid-parent Heterosis is positive and mainly significant, while their magnitude is not high enough to be useful in the prediction of Heterotic results. The number of crosses to be performed and the progeny to be screened can be reduced considerably. Marker-assisted selection (MAS) is based on the concept that the presence of a gene can be inferred from a marker which is closely associated with the gene. With MAS, the breeder can perform several selection rounds in one year, without relying on the pathogen’s natural occurrence. Encouraging results for maize streak resistance virus and drought tolerance have been obtained at CIMMYT in MAS (Ribaut et al., 2001, 2007). The availability of tightly linked genetic markers for resistance genes will help to identify plants carrying these genes at the same time without subjecting them to early generation pathogen attacks. Most of the published paper end with a conclusion that MAS would be useful, but it has not yet published the results of effective MAS effort. Structure of maize genome makes direct genome sequencing very difficult for gene discovery. DNA markers have provided useful resources in a number of analyses, from phylogenetic analysis to positional gene cloning. 50 percent of the world’s agricultural productivity has been achieved through traditional plant breeding. Biotechnology is becoming increasingly important in agriculture, but it should not be forgotten that plant breeding still plays a key role, says Gai et al. (2000). The development of high-density molecular maps facilitated by DNA markers has made mapping and tagging of nearly every trait possible and serves as bases for assisted marker selection, they say. The research will help to elucidate gene function, gene regulation and expression. Maize genome sequencing would help to elucidate gene function, gene regulation and expression. Modern biotechnologies could complement and boost the efficiency of conventional breeding and selection techniques to improve the agricultural productivity.

Major constraint for maize self-sufficiency in Nepal

Hybrid maize plays an important role for the self-sufficiency of maize in Nepal. Low seed production, diseases and plagues, management activities, socio-economic factors, etc. are among the major constraints. Different diseases and pests have been reported throughout Nepal attacking maize in Maize Fields as well as in Stores. Turcicum leaf blight is widespread in hill habitats and can cause significant losses if there is no strong genetic resistance to the variety. The banded leaf and sheath blight (Rhizoctonia solani) increased in all habitats. Downy mildew (Perona sclerospora spp.) and leaf firing in the terai; are the important diseases mentioned by farmers. White grubs, stem borers, and termites were major maize field insects. Farmers lack knowledge on the fertilizer dose and method of application. Pot also causes significant yield losses worldwide with an average of 12.8 percent amid applications for cannabis control, and 29.2 percent for non-drug control. Experimental results at ARS, Pakhibas showed weedy environment resulting in maize yield reduction of up to 70 percent. In Nepal the total irrigated area is only about 1331 thousand ha (MOAD, 2016, 2017). The formal sector’s contribution is less than 10 percent and efficiency is a productivity constraint. Large volumes of low-quality hybrids and other seeds are imported from India and distributed through agro-vets to farmers. Nepal has occasional crop failure in the past. Two-thirds of the maize is produced during the summer season in the mid hills and high hills. Delay in monsoon during planting, uneven rainfall distribution and prolonged drought during crop season can adversely affect crop yield. The lack of agricultural labor adversely affects production. NARC is suffering from an insufficient operating budget as a consequence of this also affecting maize research. These all of the above factors directly and indirectly affect maize production. If all these obstacles are resolved we will surely fulfill our goal of self-sufficiency.

Importance of single cross hybrid in present scenario of Nepal

Maize requirements are rising day by day, whether for food or for meat. Nepal requires 2750 t of feed per day for the poultry (yellow maize) industry. We consume 0.5 M t of maize grain per year, 80 percent of which (value USD 10 million) is imported from India (NFA, 2018). There are approximately 127 feed mills (2 to 400 t/day capacity) registered and engaged in Nepal’s feed industry. In the last five years, demand for poultry and animal feed has risen by around 13 percent and 8.5 percent per year, respectively. All of these above data and facts reveal the current maize consumption scenario in Nepal that clearly shows the need for higher production. So SCH comes into practice when
the case of higher demand has come in. In Nepal, the registered hybrids developed are single cross hybrids that contribute greatly to increased yield. Annual demand for maize seed in Nepal is 19552 MT. NARC-developed hybrids have yet to be marketed due to involvement in restricted market growth activities. There are many constraints in maize production in Nepal as mentioned above. To meet the demand we import seeds from foreign countries. All the hybrids created by NARC since now have been single cross hybrids (Table 2). Due to the problem in flowering synchronization, First developed hybrid of Nepal i.e., Gaurav hybrid is in the process of de-notification from the released/registered list of Seed Quality Control Center (SQCC). Maize seed distribution and adoption in Nepal Hybrid (SCH) maize has been reported to cover < 10 per cent of the total maize region (SEAN 2017; SQCC 2018). This means we've got a long way to go. The hybrids should be commercialized. Extension workers should make the pros and cons of hybrid maize clear to farmers. NARC will step up research work to develop and disseminate higher yield, pest-resistant hybrids.

**Conclusion**

Genetic enhancement is the most preferred and effective means through which farmers and environment friendly varieties can be developed in sustainable way. Identification and categorization of maize Heterotic groups through analyzing the combining ability is vital to explore Heterosis in maize. Modern plant breeding techniques and biotechnological approaches are the penetrating points for rapid progress in plant breeding. Identifying and collecting the economically and environmentally important genes is now possible within a short period of time through biotechnological tools. Comparing with other nations, Nepal is very far behind exploiting Heterosis in maize crop and using the very few biotechnological tools to enhance the conventional crop breeding. Therefore, focus should be given to explore the potential of maize genetic resources and Heterosis. Demand of maize grain is increasing mainly due to changing food consumption patterns and increased feed industry but still, large yield gap is persistent in maize crop resulting higher import of maize grain from other countries. Mainly due to the yield advantage, among the different types of maize hybrids, single cross maize hybrids cultivation in potential areas considering crop management practices is the best way to increase productivity thereby achieving the maize self-sufficiency in Nepal.

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