International Journal of Life science & Pharma Research

A REVIEW ON BIOTECHNOLOGY, GENETIC DIVERSITY IN CUMIN (*CUMINUM CYMINUM*)

MADHURI PARASHAR, M L JAKHAR AND C.P.MALIK

School of Life Sciences, Jaipur National University, Jaipur, Rajasthan, India

ABSTRACT

Cumin (*Cuminum cyminum* L.) is a significant seed spice and one of the earliest known minor spices. It is an aromatic plant included in the Apiaceae family and is used to flavor foods, added to fragrances, and used in medicinal preparations. Its fruit, known as cumin seed, is yellow to brownish-gray in color. Biotechnological tools such as molecular markers are one of the effective tools, which facilitate the plant breeder to develop new varieties and breeding strategies for crop improvement programmes.

Key Words: *Cumin cyminum*, seeds, aromatic, Apiaceae, explants, molecular.

1. INTRODUCTION

Cumin commonly known as "Jeera" generally used in the household work as a spice**.** It is a small annual herbaceous plant that is a member of the aromatic plant family (Apiaceae). Seeds of the plant are used to add flavour to spicy dishes. Cumin seeds contain numerous phyto-chemicals that are known to have antioxidant, carminative and anti-flatulent properties. The active principles in the cumin may increase the motility of the gastro-intestinal tract as well as increase the digestion power by increasing gastro-intestinal enzyme secretions. This spice is an excellent source of minerals like iron, copper, calcium, potassium, manganese, selenium, zinc and magnesium. It also contains very good amounts of B-complex vitamins such as thiamin, vitamin B-6, niacin, riboflavin, and other vital anti-oxidant vitamins like vitamin E, vitamin A and vitamin C. The seeds are also rich source of many flavonoid phenolic anti-oxidants such as carotenes, zeaxanthin, and lutein.

2. HISTORY

Cumin is native to Egypt and has been cultivated in the Middle East, India, China and Mediterranean countries for millennia. Throughout history, cumin has played an important role as a food and medicine and has been a cultural symbol with varied attributes. Cumin is mentioned in the Bible not only as a seasoning for soup and bread, but also as a currency used to pay tithes to the priests. In ancient Egypt, cumin was not only used as a culinary spice, it was also an ingredient used to mummify pharaohs. Cumin seeds were highly honored as a culinary seasoning in both ancient Greek and Roman kitchens. Cumin's popularity was partly due to the fact that its peppery flavor made it a viable replacement for black pepper, which was very expensive and hard to come by. Cumin was also noted for both its medicinal and cosmetic properties. Its application to induce a pallid complexion was frequently employed by many students trying to convince their teachers that they had pulled "all-nighters" studying for their classes. Although a much prized spice, cumin became a symbol of frugality and greed in ancient Rome. Both Marcus Aurelius and Antoninus Pius, emperors with a reputation for their avarice, were given nick names that included reference to cumin.

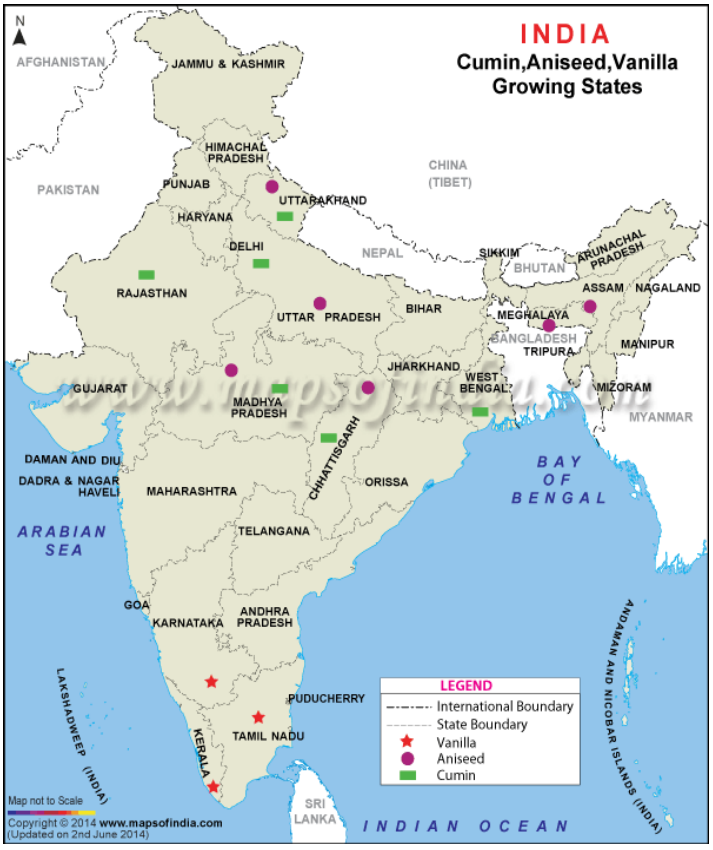

Figure 1
Map showing distribution of Cumin, Aniseed and Vanilla in India.

3. BOTANY

Cumin 'seeds' belong to family of small scented herbs known as Apiaceae. The cumin plant grows to 30-50 cm (0.98-1.6 ft) tall and is manually harvested. It is an herbaceous annual plant, with a slender branched stem 20-30 cm tall. The leaves are alternate, simple or compound and have a sheathing leaf base below (Fig 2). The flowers are small, pink and characteristically borne in umbels or umbrella-like clusters (Fig 2), where a large number of flowers with stalk of equal length spring from a common point so that all of them bloom at the same level. The flowers have both male and female structures together and an inferior ovary that develops into a very characteristic fruit called a cremocarp (Fig 2). This is dry, capsular and invariably breaks at maturity into two one-seeded bits, with a ribbed wall that has a number of longitudinal oil canals (Fig 2). It is these latter that give the characteristic odour, flavour and the very value to the fruit itself. Though these grain-like fruits are called the seeds, the true seeds are within them and come out only during germination through disintegration of the fruit wall. Cumin seeds resemble caraway seeds, being oblong in shape, longitudinally ridged and yellow-brown in colour, like other members of the Apiaceae family such as caraway, parsley and dill.

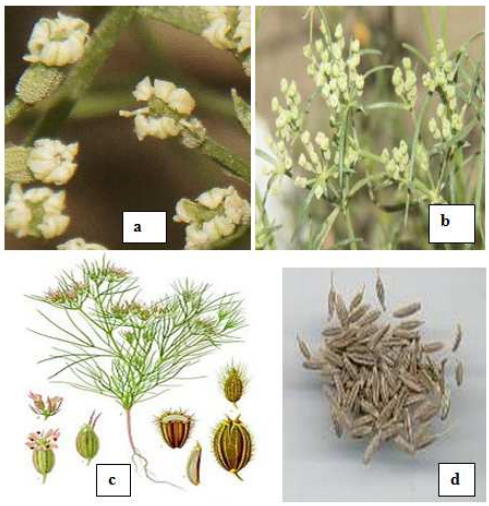

Figure 2

(a) Cumin umbels have bracteoles at their base. (b) Cumin plants have a suberect habit and inflorescences are compound umbels; the sub-umbels are borne by rays of different length.(c-d) Cumin cyminum. And 'seeds'.

4. PHYTOCHEMISTRY AND MEDICINAL PROPERTIES

Cumin seeds contain up to 5% of a volatile oil composed primarily of aldehydes (up to 60%). In addition, the seeds yield about 22% fats, numerous free amino acids, and a variety of flavonoid glycosides, including derivatives of apigenin and luteolin. The cuminaldehyde content varies considerably, depending on the source of the oil (fresh vs ground seeds). Fine grinding of the seed can result in the loss of up to 50% of the volatile oil with the greatest loss occurring within 1 hour of milling. Monoterpene hydrocarbons are another major component of the oil; sesquiterpenes are minor constituents (Ahmad and Saeidnia**,** 2011)**.** The head components of the characteristic aroma of unheated whole seeds are 3p-menthen-7al and cuminaldehyde in combination with other related aldehydes. Cumin also contains safrole, a mutagen, which is degraded by cooking. Antimicrobial activity has been reported from the volatile oils and aqueous extract of Cumin. Cumin seed oil and alcoholic extract inhibited the growth of *Klebsiella pneumonia* and its clinical isolates by improvement of cell morphology,capsule expression and decreasing urease activity.Cuminaldehyde (Fig. 3-1) is the main active compound of Cumin for this property (Derakhshan et al. 2008 and Derakhshan *et al.,* 2010). Limonene (Fig.3-2), eugenol (Fig 3-3), α- and β-pinenes(Fig. 3-4, 5) and some other minor constituents have been found in cumin oil and suggested as the active antimicrobial agents (Johri,2011 and Dorman and Deans,2000). The Cumin oil is reported as a high antioxidant mainly due to the presence of monoterpene alcohols (De Martino et al. 2009). The presence of phytoestrogens in Cumin has been reported which is related to its anti-osteoporotic effects. Methanol extract of Cumin showed a significant reduction in urinary calcium excretion and increase of calcium content and mechanical strength of bones in animals (Shirke et al. 2008). Furthermore, the aqueous extract of Cumin seeds indicated the protective effect against gentamycin-induced nephrotoxicity, which decreased the gentamycin-induced elevated levels of serum urea and enhanced the clearance of the drug (Mahesh CM et al. 2010). Anti-epileptic activity of cumin oil was also reported, which decreased the frequency of spontaneous activity induced by pentylene tetrazol (PTZ) (Janahmadi M et.al. 2006). Recently, Cumin oil has been found to act as a significant analgesic by formalin test in rats and suppress the development and expression of morphine tolerance and also reverse the morphine dependence (Koppula et al. 2009 and Haghparast et al. 2008 and Khatibi et al. 2008). Other important reports consider that dietary Cumin can inhibit benzopyrene-induced for stomach tumorigenesis, 3-methylcholanthrene induced uterine cervix tumorigenesis, and 3-methyl-4-dimethyaminoazobenzene induced hepatomas in mice, which was attributed to the ability of Cumin in modulating carcinogen metabolism *via* carcinogen-xenobiotic metabolizing phase I and phase II enzymes (Gagandeep et al. 2003).

Literature review on phytochemistry of the Cumin seeds revealed the presence of various bioactive compounds, the important secondary metabolites of which are discussed as followed (Takayanagi et al. 2003 and Kitajima et al. 2003). Two sesquiterpenoid glucosides, cuminoside A and B (Fig.3- 6 and 7),and two alkyl glycosides (Fig.3- 8, 9) were isolated together with some known compounds from the methanol extract of Cumin seeds. Their structures were established as (1*S*,5*S*,6*S*,10*S*)-10-hydroxyguaia-3,7(11)-dien-12,6-olide β-D glucopyranoside (Fig.3-6),(1*R*,5*R*,6*S*,7*S*,9*S*,10*R*,11*R*)-1,9-dihydroxyeudesm-3-en-12, 6 olide 9-*O*-β-Dglucopyranoside (Fig.3-7), methyl β-D-apiofuranosyl-(1→6 β-D-glucopyranoside (Fig.3-8) and ethane-1,2-diol 1-*O*-β-D-apiofuranosyl-(1→6) β-D-glucopyranoside (Fig 3.-9) (Takayanagi et al. 2003). In another report, three glycosides (Fig.3), 1-*O*-β-Dglucopyranoside (Fig.3-10), 3-*O*-β-D-glucopyranoside (Fig.3-11) and 4-*O*-β-D-glucopyranoside (Fig:3-12) have been isolated and structural elucidated from the seeds (fruits) of Cumin (Kitajima et al. 2003).Mediterranean climates. It is grown from seed, sown in spring, and needs fertile, well-drained soil. The plant blooms in June and July. The seeds are normally ripe four months after planting. The plants are threshed when the fruit is ripe and the seeds are dried. (Evans,1997 and Kafi, 2002).

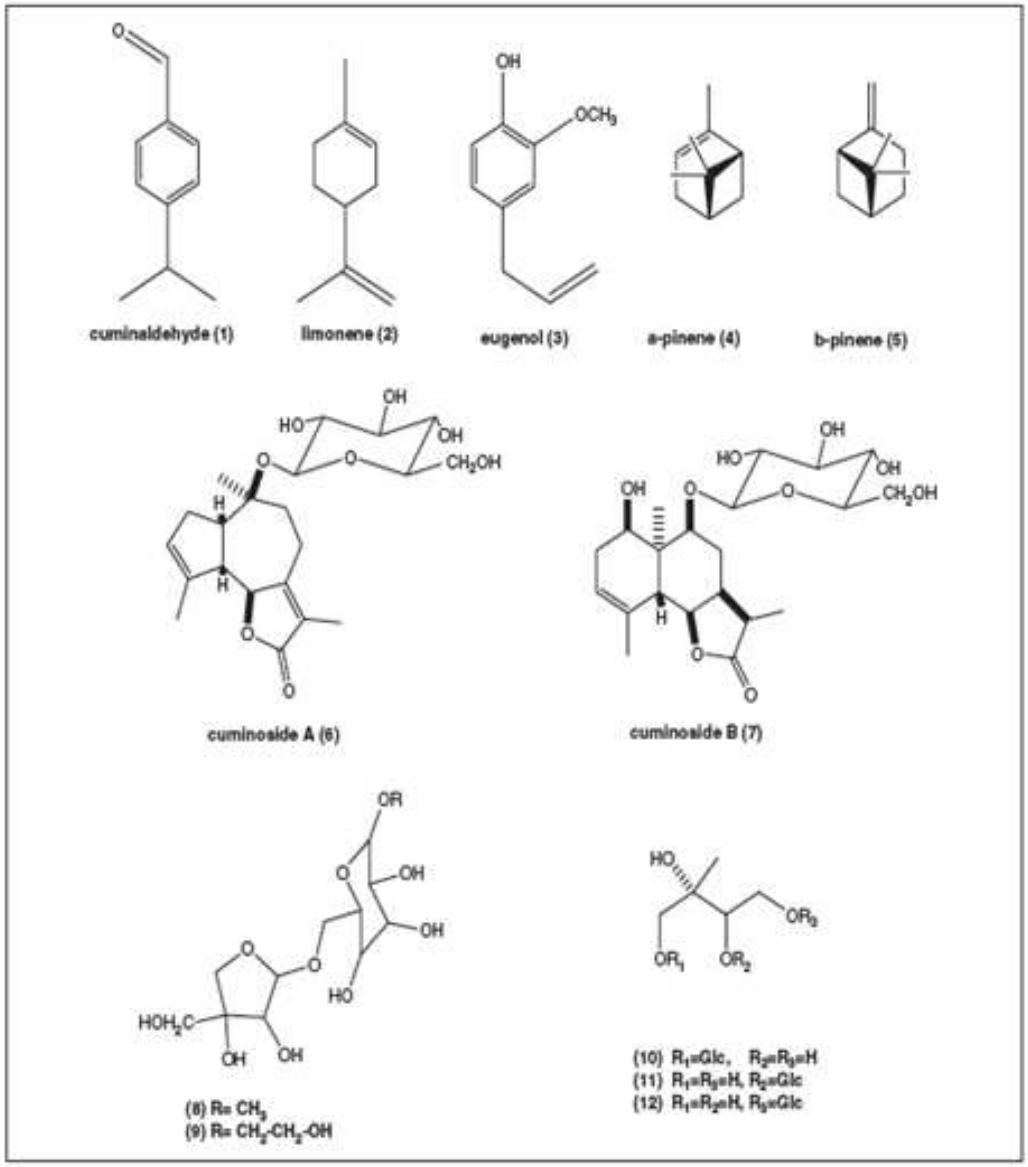

Figure 3

Chemical structure of the isolated compounds from Cumin (Adopted from Pharmacognosy Journal, 2011 3 (25)).

5. HEALTH BENEFITS

Cumin occupies the stellar ranks of Indian, Middle Eastern and Mexican cooking. It has tremendous health benefits.

- Iron for energy and immune function
- Seeds for good digestion
- Cancer prevention
- Respiratory disorders, asthma, bronchitis
- Insomnia
- Piles
- Lactation
- Boils

6. TISSUE CULTURE STUDIES

A in*vitro* protocol was developed for regeneration of adventitious cumin shoots from explants of hypocotyl and stem internodal segments. The best response towards shoot induction was obtained from explant tissues of stem internodes when placed slightly embedded (3–4 mm) in Murashige and Skoog (MS) solid medium containing 2.5 µM benzyladenine (BA). Shoot initiation was absent on medium lacking BA. The induced shoot initials on BA-containing medium proliferated and elongated during secondary culture on the medium lacking plant growth regulators (PGR). Up to 30 shoots per explants were harvested on this medium. Although, these shoots readily formed roots (95%) on PGR-free medium, only 30% of them survived after the acclimatization to the greenhouse conditions. The plant survival rate *ex vitro* was enhanced (73%) when the excised shoots were cultured for rooting in the medium with 2% polyethylene glycol (PEG 6000). Adding 1 µM indole-3- butyric acid (IBA) to the PEG-containing medium increased the percentage of rooting from 75 to 85%. (Azza et al.2001). Hypocotyls was induced from callus and primary leaf explants of cumin (*Cuminum cyminum* L.) seedlings on a medium with 4 µM 2,4-D alone or plus 2 or 4 µM kinetin. An embryogenic callus developed within 2 weeks after transferring the callus to medium lacking plant growth regulators (PGR). The presence of kinetin in the callus induction medium with 2,4-D enhanced both the callus proliferation and the subsequent differentiation of the embryoids on the PGR-free medium. Plumules with or without simultaneously developed roots were observed 3–4 weeks after subculturing the embryogenic callus on medium containing 0.5 or 1.0 µM kinetin. Subsequently, they were transferred onto half-strength medium supplemented with 1 µM indole-3-butyric acid (IBA) and 2% polyethylene glycol (PEG, 6000) for root induction and/or proliferation, and *in vitro* hardening of the regenerated plants. The survival rate *ex vitro* was 70%. No plants developed from the embryogenic callus continuously incubated on medium lacking kinetin. We concluded that kinetin is crucial for plant regeneration from the induced embryoids of cumin (Azza *et al.,* 2002). A new, efficient method was developed for multiple shoot regeneration of cumin from imbibed embryo cultures. This method yielded a large number of shoots within short period of time (30–50 days) without any sub culturing. The effects of different media, different embryo explants and various combinations of plant growth regulators (PGRs) on callus formation and shoot regeneration in cumin were investigated. Simultaneous callus formation and shoot regeneration was obtained. The best response for multiple shoot regeneration was observed on B5 medium containing 1.0 mg l^{-1}BAP, 0.2 mg l^{-1} NAA and 0.4 mg l^{-1} IAA, with an average of 140 shoots per explant (Esmaeil et al. 2003).

After embryo culture, shoots with single-cellular origin were regenerated from the meristematic zone of embryo without any intermediate callus phase. In contrast, proliferated shoots with multi-cellular origin were directly regenerated from the axillary buds (meristems) of node explants. Effects of different concentrations of 6-Benzylaminopurine (BAP), alpha-Naphthaleneacetic Acid (NAA) and Indole-3-kcetic Acid (IAA) on B5 medium of embryo and node cultures as well as subculture were studied in detail. In direct organogenesis pathway from

embryo explants, 0.1 mg L(-1) NAA + 1 mg L(-1) IAA resulted the highest shoot regeneration response (89.5 shoots per regenerated explants), whereas 0.1 mg L(-1) BAP + 1 mg L(-1) NAA was the most effective combination in direct shoot proliferation from node explants (42 shoots per regenerated explants). BAP (cytokinin) revealed the inhibitory effect on induction of direct shoot organogenesis pathway from embryo explants, while low concentration of BAP (0.1 mg L (-1)) had positive effect on direct shoot proliferation pathway from node explants. Sub culturing was not necessary for shoot multiplication and elongation in embryo culture, whereas multiplication and elongation of shoots in node culture were associated to subculture on growth regulator-free medium. In other part of study, the behavior of different cumin genotypes in direct regeneration pathways was studied. Both direct organogenesis and direct proliferation pathways were applicable to different cumin genotypes and regenerated plants were phenotypically normal. This study supports the feasibility of combined direct regenerations protocols from embryo and node of cumin in germplasm conservation by in vitro cloning and genetic improvement programs. (Ebrahimie et al. 2007).Foscate (1979) worked out several factors which regulated morphogenosis of Cumin plants. Bhojvani and Radian (1996) reported callus formation from hypocotyls explants after two weeks on 2,4-D.Shukla et al. reported similar results and found the callus has green and semicompact. Shukla et al. (2010) reported embryogenic callus drive from cotyledon of cumin and obtain heart and torpedo shape embryo which fail to germinate into plantlets. In a recent publication Jakhar et al.(2013) described in vitro regeneration and characterization of in vitro mutants in Cumin. These authors found that the shoot apex and hypocotyls explants responded differently to various concentration of cyto kinin and auxin used alone or in combination to induce callus formations.these authors standerzied concentration of BAP or callus induction in hypocotyls driv callus globular embryo like structure were observed with 2,4 –D but this callus fail to regenerate in full fledged plant. Regenerated shoots formed roots within three weeks after culturing on NAA the callus was subjected to X-ray treatment raised on M.S. medium supplemented with BAP. The nature of mutant was characterized through SDS / PAGE of leaf protein.

7.PHARMACOLOGICAL PROPERTIES OF CUMIN

- Anti-microbial
- Anti-diabetic
- Anti-cancer
- Anti-oxidant
- Anti-osteoporotic
- Immuno modulatory
- Gastrointestinal disorders
- Central nervous system
- Anti-asthmatics
- Skin disorders & boils
- Ophthalmic effects

8. MOLECULAR STUDIES

Genetic diversity of cumin and determine the traits effective on seed yield and cumin aldehyde has been investigated. The diversity has been studied based on phenotypic and biochemical characteristics. In all forty nine cumin ecotypes constituting sub-populations belonging to nine populations from different provinces of Iran were assessed. Results indicated a significant variation for all the considered traits among and within populations derived from different provinces. Kerman and Esfahan populations showed the best performance based on the phenotypic data, while Yazd had almost the lowest levels of traits. Correlation analysis showed number of seed per umbel and umbel per plant had highest relationship with seed yield. Path analysis also demonstrated that number of umbel per plant and number of seed per umbel had the most direct effects on seed yield and were identified as the most effective factors on seed yield. Cumin aldehyde was mostly correlated by number of umbel per plant. The study showed that different qualitative characteristics such as seeds with light color and without trichome and leaves without trichome, vary and large pods of petiole tended to produce high seed yield. Pattern analysis of different populations based on first two main principal components categorized the measured genotypes in to three groups: Pars, Northern-Khorasan, Golestan, Semnan and Yazd (Group1), Southern-Khorasan and Khorasan-Razavi (Group2) Kerman and Esfahan (Group3), which the third group are high yielding genotypes with different genetic background could be advised for cultivation and breeding programs. So the

available genetic diversity among the Iranian cumin populations could lead to produce high yielding population of cumin (Alireza et al. 2011). In a subsequent paper Alireza et al. (2012), undertook detail research on genetic diversity; forty nine cumin ecotypes, belonging to nine Iranian regional sub-populations were evaluated using RAPD markers. DNA was extracted through CTAB method. Twenty three RAPD markers were used for diversity assessment, in which 21 showed polymorphism. Allele frequency and polymorphism information content (PIC) of each locus were calculated by Power Marker version 3.25. Molecular variability among and within populations was assessed accordingly. Based on molecular data, Jacquard's similarity coefficient was used to detect the phylogenetic relationship; subsequently dendrograms were drawn based on UPGMA using NTSYS software. Cluster analysis among the populations categorized nine populations into two groups at the similarity level of 0.43, in which class one was consisted of only Golestan population and the rest were arranged in the second group. Golestan and Northern-Khorasan populations showed the highest difference while Kerman and Esfahan populations showed the most similarity. Based on Principal Coordinate Analysis (PCOA), 79% of variation was explained by two first principal components. Populations of Semnan, Yazd and Golestan showed different reaction rather than the other populations. It was suggested that Kerman, Esfahan and Southern-Khorasan may have the same ancestors. Molecular diversity among 49 ecotypes, as the sub-populations of nine populations derived from different Iranian states, showed five categories. Based on these data it was inferred that there was a high potential of variability in Iranian cumin populations which are very important sources for cumin breeding objectives. (Alireza et al. 2012)**.**

Genetic variation among 42 cumin accessions were collected from different regions of Iran plus two accessions from Syria and Afghanistan were assessed based on three marker systems namely, ISSR, RAPD and morpho-agronomic traits. In overall, banding patterns of 22 ISSR primers and 13 RAPD primers revealed 202 (67.32%) and 85 (54.90%) polymorphic bands, respectively. The range of similarity coefficient in ISSR and RAPD markers were 0.48-0.92 and 0.25-0.94, respectively. Using primers as pairwise combination in this study did not offer higher polymorphism but provided different band pattern. Specific grouping were carried out by each cluster analysis including ISSR, RAPD, ISSR+RAPD and morpho-agronomic markers based on their similarity matrix making 8,7,6 and 3 groups respectively. The results showed that grouping based on molecular markers and morpho-agronomic traits are different so these two systems could not discriminate accessions as a same way. All of Mantel tests between extracted similarity matrices from each marker system were significant except between ISSR marker and morpho-agronomic traits. It could be concluded that among three different molecular data sets, the RAPD and RAPD+ISSR data have a significant and closer relationship to morpho-agronomic data. **(** Hossein et al. 2013). Iran is one of the most important centres of genetic diversity because of variable climates, old civilization, and Kerman province having ecological diversity has unknown potential that must be considered sufficient. Green cumin is an endemic plant with plenty of medicinal properties and unknown genetic diversity that has been considered in this research. In this study, 32 genotypes of *Cuminum cyminum* containing 29 samples of different area from Kerman province and three samples from townships of Sabzvar, Tabas and Birjand were assessed by RAPD molecular markers.DNA extraction was done by modified CTAB method. After DNA extraction stages, complement gene loci were amplified by 15 RAPD primers. These primers produced 154 bands, that 133 bands (about 86%) were polymorphic. Cluster analysis based on the resulting data was performed using UPGMA method and Dice's similarity coefficient in NTSYS software. The resulting dendogram categorized the accessions into 6 groups in 46% similarity**.** Genetic diversity in plant inherited stores in order to classify the genotypes regarding resistance to biotic and abiotic stress, preventing from genetic erosion is one of the basic and fundamental steps in the most breeding programs.(Amin et al. 2013).

8.1 GENETIC DIVERSITY USING MOLECULAR MARKERS

According to our study, Genetic diversity was carried out in 12 varieties by using RAPD, ISSR, SCoT and CCMP markers. A total of 100 ISSR primers, 400 RAPD primers, 36 SCoT primers and 10 CCMP primers were used for the analysis. Total genomic DNA was extracted from young

leaves following the standard CTAB method with minor modifications.

8.1.1 RAPD *ANALYSIS*

Out of 400 primers, 56 primers were amplified. Of these, 55 were polymorphic and 1 was monomorphic. The number of bands amplified per primer varied between 2(OPR10) and 19 (OPD-20) with an average of 10.5 bands per primer. A total of 508 bands were amplified of which 321 were polymorphic resulting in a polymorphism frequency of 63.18% and an average of 5.8 polymorphic bands per primer. similarity matrix values using Jaccard coefficient based on RAPD markers ranged from 0.37 between accessions 5 and 9 to 0.87 between accessions 7 and 8.Dendrogram showed two major clusters smaller one having one variety 9 and the larger cluster that could be further divided into 8 different sub clusters.

Table 1

shows the list of RAPD Primers with their respective number of bands, No. of polymorphic bands and % polymorphism.

Sr.no.	Primer	Total no of bands	No of polymorphic bands	Polymorphism (%)
1	OPA 1	13	11	84.61
2	OPB 2	12	10	83.33
3	OPB 3	14	11	78.57
4	OPB 9	10	8	80
5	OPD 17	6	5	83.33
6	OPD 20	19	13	68.42
7	OPE 3	6	5	83.33
8	OPE 7	13	8	61.53
9	OPE 14	8	6	75
10	OPE 18	4	2	50
11	OPF 15	12	8	66.66
12	OPF 20	10	7	70
13	OPG 4	6	3	50
14	OPG 18	9	7	77.77
15	OPH 5	6	3	50
16	OPH 11	12	9	75
17	OPH 13	11	9	81.81
18	OPH 16	7	5	71.42
19	OPH 17	3	1	33.33
20	OPI 2	17	13	76.47
21	OPI 3	6	5	83.33
22	OPJ 4	6	4	66.66
23	OPJ 5	6	5	83.33
24	OPJ 7	5	4	80
25	OPJ 10	4	2	50
26	OPJ 14	14	13	92.85
27	OPJ 20	5	4	80
28	OPK 7	4	3	75
29	OPK 19	6	5	83.33
30	OPL 7	18	16	88.88
31	OPL 12	16	15	93.75
32	OPL 16	5	3	60
33	OPM 2	13	10	76.92
34	OPM 11	12	11	91.66
35	OPM 19	11	6	54.54
36	OPN 2	4	3	75
37	OPN 5	9	5	55.55
38	OPN 10	4	3	75
39	OPO 3	6	5	83.33
40	OPO 7	10	7	70
41	OPO 12	11	9	81.81
42	OPP 10	3	2	66.66
43	OPP 12	15	12	80
44	OPP 14	15	14	93.33
45	OPP15	13	8	61.53
46	OPP16	9	4	44.44
47	OPP17	10	6	60

48	OPP 19	8	3	37.5
49	OPQ 3	9	7	77.77
50	OPQ 5	3	1	33.33
51	OPQ 13	7	6	85.71
52	OPQ 14	12	8	66.66
53	OPR 6	16	13	81.25
54	OPR 10	2	1	50
55	OPS 11	14	12	85.71

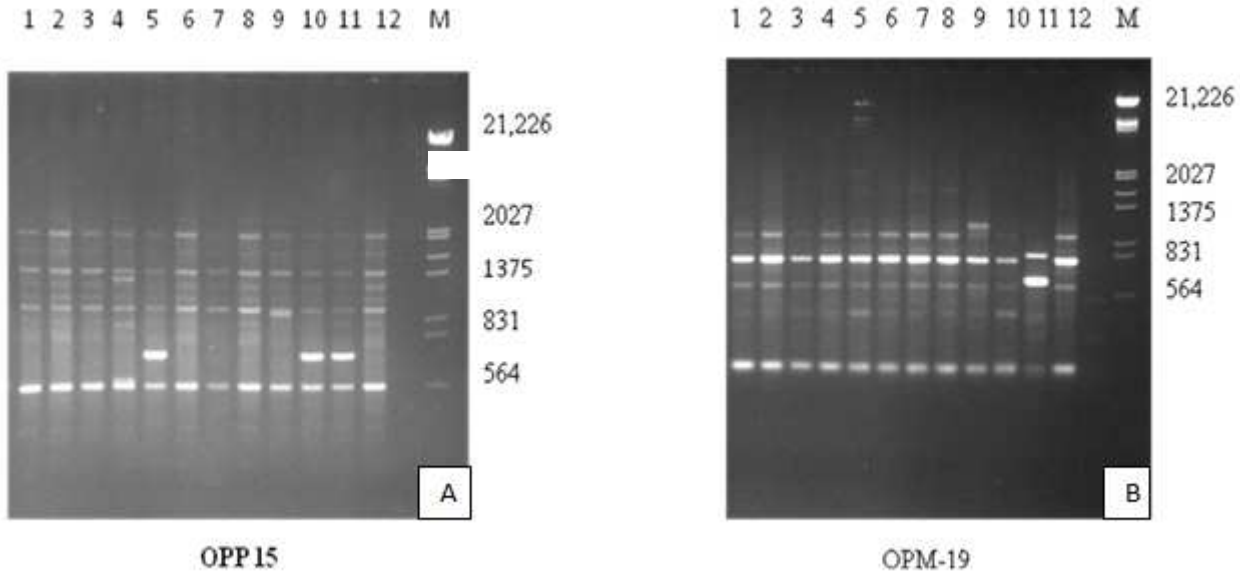

Figure 4
Analysis of 12 varieties of cumin cyminum using RAPD primers (A) OPP 15 (B) OPM-19.

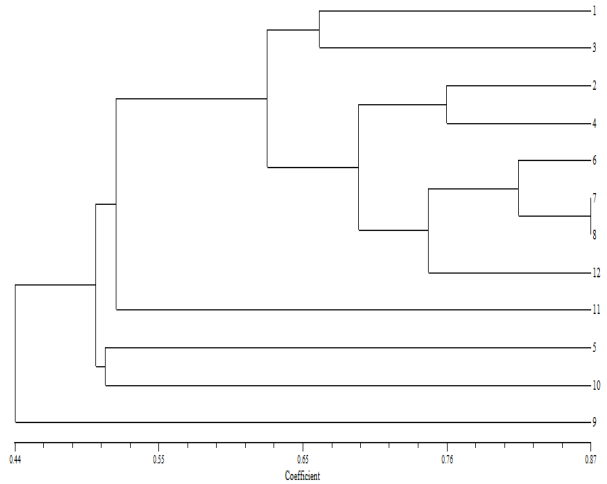

Figure 5
Shows the dendrogram constructed through NTSYS (2.02 pc).

8.1.2 ISSR Analysis

Out of 100 UBC primers, 39 primers gave amplification products. Of these 27 primers gave polymorphic bands while 12 primers were monomorphic. The number of amplicons per primer ranged from 4 (UBC 840 and UBC 852) to 19 (UBC 855). The polymorphic primers amplified 305 bands of which 169 were polymorphic resulting in a polymorphic frequency of 55.40% and an average of 6.2 polymorphic bands per primer. The polymorphism was maximum (88.88 %) with primers UBC 873, and was low (18.18 %) with UBC 817. The similarity matrix values using Jaccard's coefficient based on ISSR markers ranged from 0.63 between variety 2 and 13, varieties 4 and 10 and 0.94 between varieties 7 and

12. The dendrogram was constructed by using NTSYS software. It illustrated two major clusters, smaller one having variety 6 and the larger cluster which could be further divided into different sub clusters .

Table 2

Shows the list of ISSR Primers with their respective number of bands, No. of polymorphic bands and % polymorphism.

Sr. no.	Primer	Total no. of bands	No. of polymorphic bands	Polymorphism (%)
1	UBC 808	12	4	33.33
2	UBC 809	10	6	60
3	UBC 811	13	6	46.15
4	UBC 817	11	2	18.18
5	UBC 818	11	3	27.27
6	UBC 825	13	8	61.53
7	UBC 826	10	6	60
8	UBC 829	9	3	33.33
9	UBC 830	8	3	37.5
10	UBC 835	12	9	75
11	UBC 836	10	4	40
12	UBC 840	4	2	50
13	UBC 841	11	7	63.63
14	UBC 842	11	6	54.54
15	UBC 847	8	5	62.5
16	UBC 848	12	7	58.33
17	UBC 850	8	6	75
18	UBC 852	4	3	75
19	UBC 855	19	13	68.42
20	UBC 857	15	6	40
21	UBC 859	10	5	50
22	UBC 861	9	5	55.55
23	UBC 866	13	7	53.84
24	UBC 868	9	5	55.55
25	UBC 873	18	16	88.88
26	UBC 878	17	11	64.70
27	UBC 880	18	11	61.11

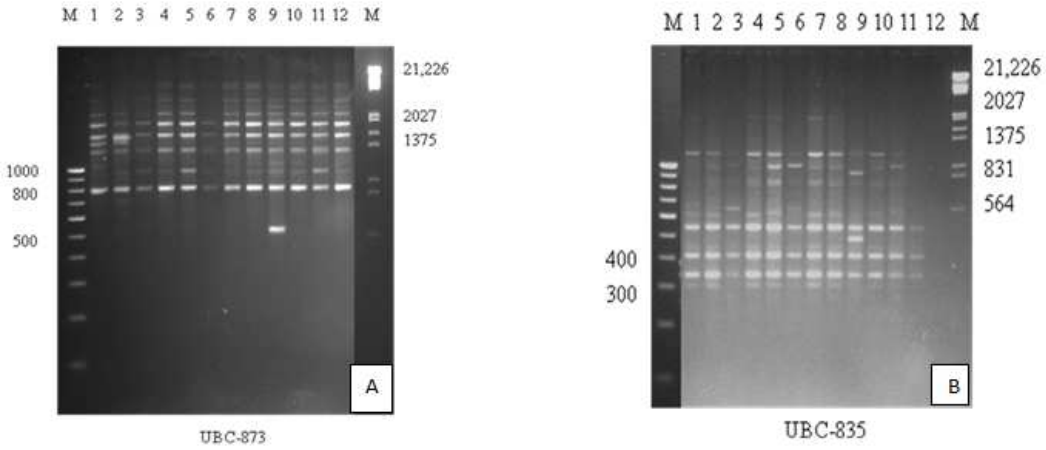

Figure 6

Analysis of 12 varieties of cumin cyminum using ISSR primer (A) UBC-873 (B) UBC-835.

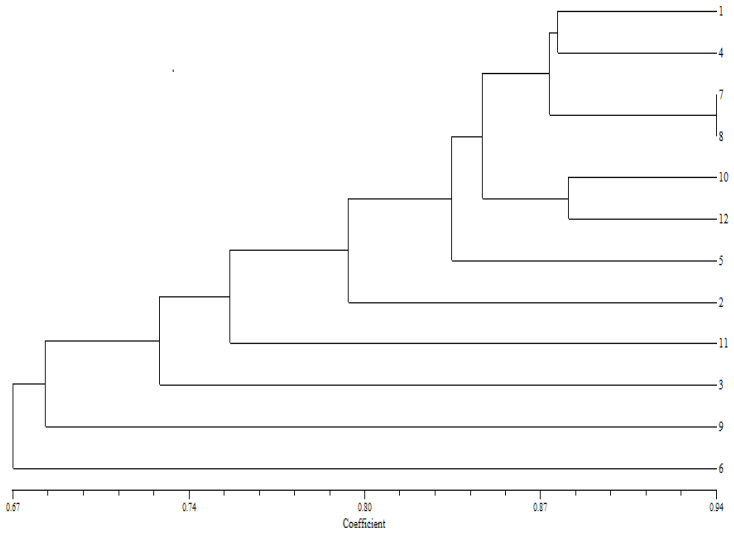

Figure 7
Shows the dendrogram constructed through NTSYS (2.02 pc).

8.1.3 SCoT Analysis

Out of 36 primers, 32 primers gave amplification products all the primers produce polymorphic bands. The number of bands amplified per primer varied between 10 (SCoT22 and SCoT31) and 35 (SCoT19, SCoT25 and SCoT28). The polymorphic primers amplified 723 bands of which 577 were polymorphic resulting in a polymorphic frequency of 79.80% and an average of 18.03 polymorphic bands per primer. The polymorphism was maximum (94.28 %) with primers SCoT25, and was low (41.17%) with SCoT 34.The similarity matrix values using Jaccard's coefficient based on SCoT markers ranged from 0.24 between varieties 1 and 11, 4 and 11 and 0.79 between varieties 7 and 8. The dendrogram was constructed by using NTSYS software. It illustrates two major clusters, smaller one having population from varieties 5 and 11 and the larger cluster which could be further divided into different sub clusters.

Table 3
shows the list of SCoT Primers with their respective number of bands, No. of polymorphic bands and % polymorphism.

Sr. no.	Primer	Total no. of bands	No. of polymorphic bands	Polymorphism (%)
1	SCoT1	25	11	44
2	SCoT2	16	10	62.5
3	SCoT3	19	14	73.68
4	SCoT4	11	6	54.54
5	SCoT5	27	21	77.77
6	SCoT6	26	24	92.30
7	SCoT7	23	19	82.60
8	SCoT8	15	14	93.33
9	SCoT9	18	16	88.88
10	SCoT10	16	15	93.75
11	SCoT11	18	11	61.11
12	SCoT12	23	19	82.60
13	SCoT13	33	28	84.84
14	SCoT14	24	20	83.33
15	SCoT15	27	23	85.18
16	SCoT16	22	18	81.81
17	SCoT17	29	27	93.10
18	SCoT18	28	20	71.42
19	SCoT19	35	28	80
20	SCoT21	31	27	87.09
21	SCoT22	10	4	40
22	SCoT23	24	19	79.16

23	SCoT24	17	16	94.11
24	SCoT25	35	33	94.28
25	SCoT28	35	32	91.42
26	SCoT30	22	21	95.45
27	SCoT31	10	8	80
28	SCoT32	11	8	72.72
29	SCoT33	22	19	86.36
30	SCoT34	17	7	41.17
31	SCoT35	35	27	77.14
32	SCoT36	19	12	63.15

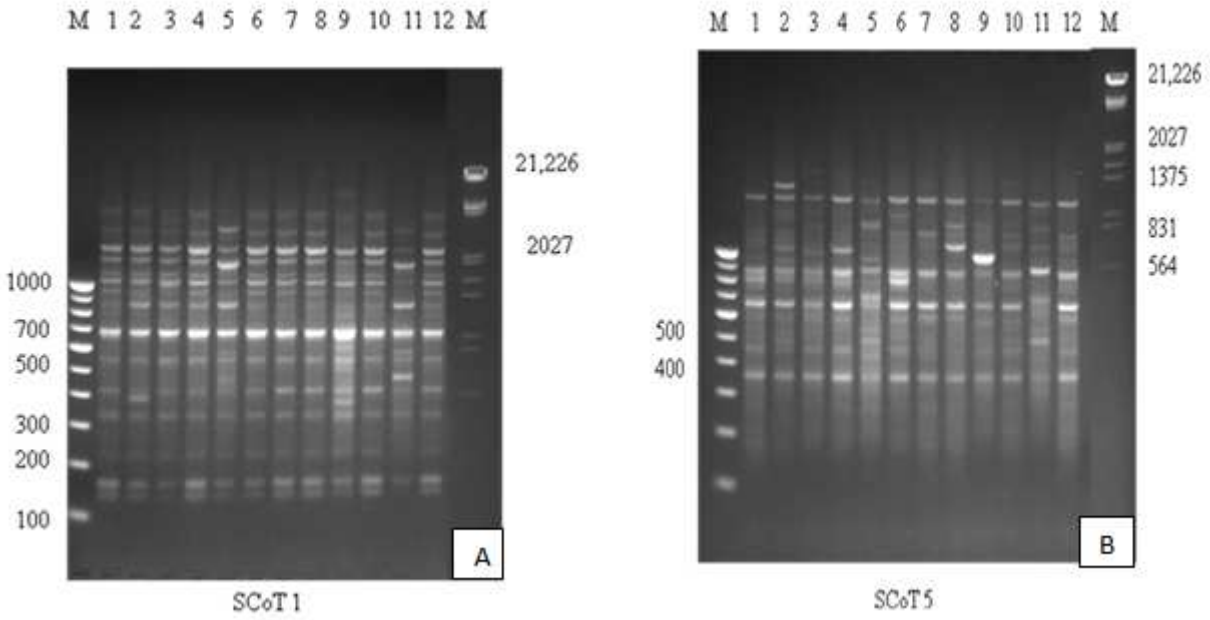

Figure 8
Analysis of 12 varieties of cumin cyminum using SCoT primers (A) SCoT 1 (B) SCoT 5.

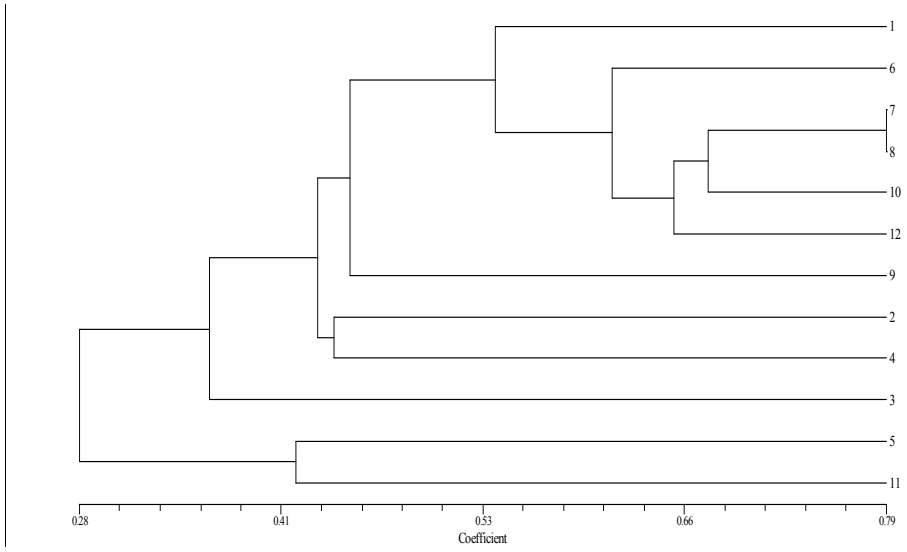

Figure 9
Shows the clusters constructed through NTSYS (2.02 pc) drawn as dendrogram.

8.1.4 CCMP Analysis

All the 10 primers gave amplification products. Of these 5 primers gave polymorphic bands while 5 primers were monomorphic. The polymorphic primers amplified 13 bands of which 7 were polymorphic resulting in a polymorphic frequency of 53.84% and an average of 1.4 polymorphic bands per primer. The polymorphism was

maximum (66.66 %) with primer ccmp 2 and ccmp 7 and low (33.33 %) with ccmp 5. The similarity matrix values using Jaccard's coefficient based on ccmp markers ranged from 0.66 to 1. The dendrogram was constructed by using NTSYS software. Dendrogram illustrate two major clusters, smaller one having variety 9 and the larger cluster which could be further divided into different sub clusters.

Table 4

Primers with their respective number of bands, No. of polymorphic bands and % polymorphism.

Sr. no.	Primer	Total no. of bands	No. of polymorphic bands	Polymorphism (%)
1	Ccmp2	3	2	66.66
2	Ccmp5	3	1	33.33
3	Ccmp6	2	1	50
4	Ccmp7	3	2	66.66
5	Ccmp10	2	1	50

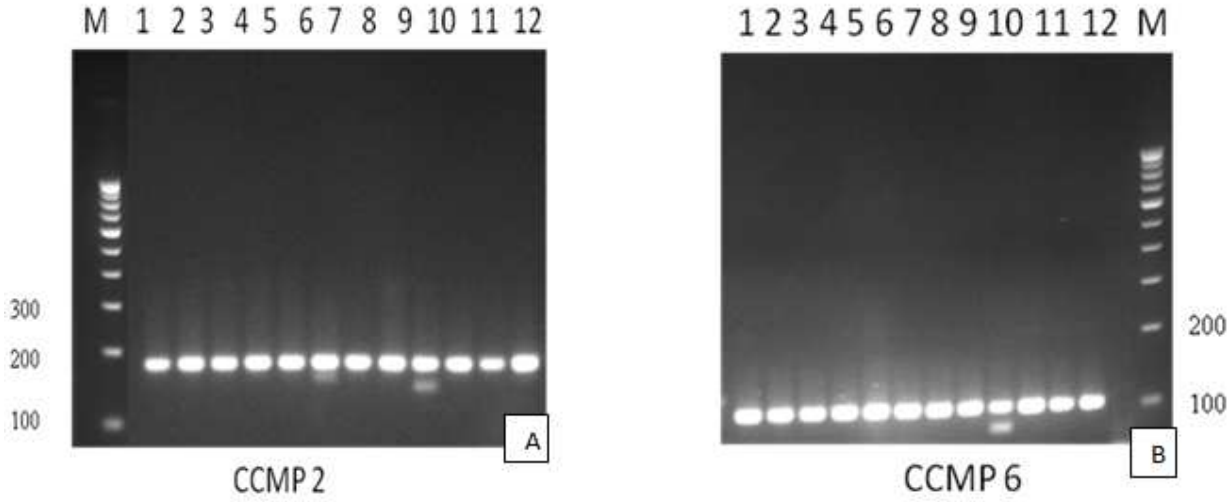

Figure 10

Analysis of 12 varieties of cumin cyminum using CCMP primers (A) CCMP 1 (B) CCMP 5.

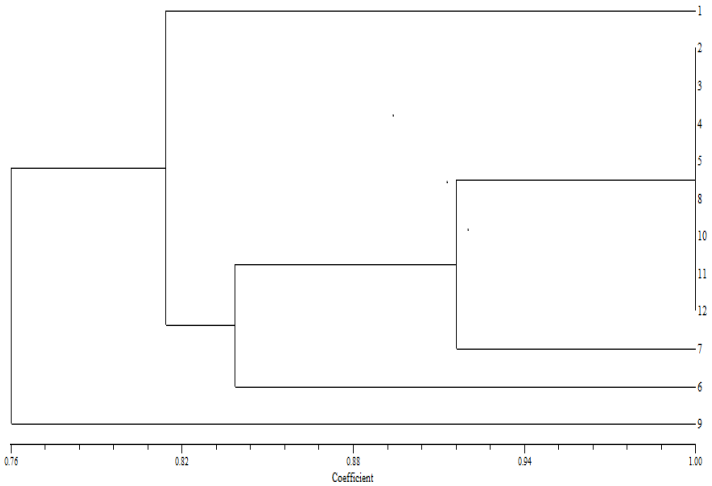

Figure11

Shows the clusters constructed through NTSYS (2.02 pc) drawn as dendrogram.

9. CROP IMPROVEMENT APPROACH IN CUMIN

Jakhar and Rajput (2013) have summarised some of the traits which deserve to be improved in this important spice and these are:

- Breeding for high yield
- Volatile oil content
- Resistance to powdery mildew
- Resistance to blight
- Resistance to wilt
- Seed size, colour and lustre
- Development of cultivars resistant to yellowing

10. BREEDING STRATEGIES

In the following some important breeding strategies for development of sustainable cultivars with high yield, resistant to abiotic, biotic stresses and desired quality are described:

- Germplasm collection, evaluation and conservation.
- Population improvement and selection.
- Exploitation of Heterosis.
- Breeding for abiotic and biotic stresses.
- Improvement of quality.
- Mutation breeding.
- Breeding varieties for mixed cropping.
- Biotechnological approaches.

10.1 GERMPLASM COLLECTION, EVALUATION AND CONSERVATION

Available literature indicates that variability in the germplasm particularly for yield, quality (volatile oil content) and reaction to different biotic and abiotic stresses is low in all the seed spices (Sharma, 1994). In the present germplasm collections variability for plant height, maturity duration is low even for these traits obviously there is an urgent need to enhance the germplasm base to successfully support the crop improvement programme. Genetic enhancement of seed spices is possible only through accumulation of variability in the form of germplasm. India is endowed with germplasm of different kind of seed spices crops and a number of indigenous and exotic origins are available at different centre's (Table) Germplasm collection activities were initiated from Rajasthan and Gujarat, the major areas for the cultivation of cumin. There is tremendous scope for collection of valuable land races of seed spices. The development of improved cultivars has improved the production and productivity of seed spices to some extent, which has endangered the accumulated diversity among the traditional cultivars of these crops species. Therefore, the conservation of germplasm of the seed spices is of profound importance for sustaining their production. If not collected and conserved these valuable genetic resources may be lost forever, hence prime importance is required to be given in collection and conservation of the biological diversity of seed spices from all over the country and abroad.

Table 5
Germplasm collection of cumin in India.

Cumin	Germplasm lines maintained		Total
	Indigenous	exotic	
In Jobner, Rajasthan	275	1	276
In Jagudan,Gujrat	240	7	247
NRCSS,Ajmer,Rajasthan	77	7	83

Evaluation and characterization of seed spices germplasm is required for their documentation and cataloguing crop wise for the further use by plant breeders and biotechnologists for improving in respect of yield, quality and resistant against biotic as well as abiotic stresses. Proper maintenance of the germplasm collections is essential to maintain the genetic structure of the original sampled population. This can be achieved by prevention of out crossing with other species and reduction the effect of natural selection in an environment other than the original one. Seed spices have both self and cross mode of reproduction. In the self pollinated seed spices gene and genotypic composition can be preserved in the absence of selection, whereas in cross pollinated it can be maintained through controlled pollination.

10.2 POPULATION IMPROVEMENT AND SELECTION

Population improvement is the process of pyramiding of the positive genes for desirable characters in a variable population through selection or recurrent selection. The appropriate strategy in Apiacee (cross-pollinated) spices would be to have short term and long term improvement programme. The short term programme would aim to improve the elite populations through recurrent selection based on the performance of individual plant progenies or even mass-selection. This approach has produced a number of good varieties cumin (RZ- 19 and RZ-209).

10.3 EXPLOITATION OF HETEROSIS

Though recurrent selection, can be successfully employed in both intra as well as interpopulation improvement, the best approach to exploit both the additive and non-additive gene effects would be the heterosis breeding. Search for cytoplasmic – genetic types of system of controlling male sterility should be taken up to make the heterosis breeding a reality.

10.4 BREEDING FOR BIOTIC AND ABIOTIC STRESS RESISTANCE

The seed spices are affected by various diseases which reduce the yield level which is already low. Some of the diseases in cumin wilt and blight, have so far evaded the effective control measures. Breeding programmes for resistance to these diseases need to be immediately initiated. Identification of pathogen and or its races causing the diseases, developing techniques to create uniform and artificial disease epiphytotics, use of tissue culture as an aid to accelerate the resistance screening programmes are some of the aspects on which research work need to be initiated to make the resistance breeding effective. Resistance breeding is also directly related to quality of the produce as it obviates the necessity of application of pesticides which often leaves undesirable residues. In addition to biotic stresses these crops are also affected by large number of abiotic stresses, therefore screening programmes on these stresses need to be initiated eg:- drought, salt and frost tolerant cultivars.

10.5 IMPROVEMENT OF QUALITY

Quality of the produce needs special emphasis in seed spices as these are exported of foreign markets where the quality standards are very stringent. Volatile oil content, shape, size and luster of grains and their cleanliness constitute the important quality factors. Appropriate weightage has to be given to these quality attributes in the breeding programmes.

Table 6
Quality parameter in cumin.

Seed Spice	Quality parameter
Cumin	High volatile oil and high cuninaldehyde content

10.6 MUTATION BREEDING

Mutations, induces with gamma rays, are useful in creating desirable variability in Spices.

10.7 BREEDING VARIETIES FOR MIXED CROPPING

Yield fluctuations over years as well as locations are very wide. Preliminary information obtained from evaluation of varieties/ or germplasm suggested that genetic differences among varieties for stability do exist. Factors responsible for differences need to be specially identified and utilized in breeding programme. In general these crops, particularly the cumin, respond very less to

agronomic inputs *e.g.* fertilizers as well as irrigation. Attention therefore, needs to be given on identification of responsive types. The high genotype x environment interaction, noted for these crops, also suggests that breeding programmes may be targeted for specific environment and develops suitable varieties for each condition.

10.8 BIOTECHNOLOGICAL APPROACHES

Different biotechnology techniques are used as the aids for improvement of seed spices such as somaclonal variations and production of transgenic lines and molecular characterization, at least of the elite germplasm needs to be immediately taken up, to facilitate their use in crop improvement programme in following ways.

A. Use of molecular markers in germplasm collection, evaluation and conservation.

B. Identification of resistant genes using isolated pathogenic strains and pure genotypes.

C. Understanding of genetic structure of population using molecular markers.

D. Tagging of genes of economic importance including genes for resistance to biotic and abiotic resistance and quality.

E. Enhancement of germplasm through soma-clonal variants, *in vitro* selection schemes for biotic/abiotic resistance.

F. Development of various molecular markers for marker assisted selection.

It may be concluded that in seed spices, where genetic variability assembled so far has been inadequate, well planned efforts be taken up to enhance the variability. Besides the systematic collection and maintenance of indigenous germplasm, exotic material particularly from centers of origin and secondary centers of variability must be introduced.

Biotechnological approaches and mutation breeding should also be employed for enhancing, variability. Since the resistance to disease and improvement in quality has special significance in seed spices, a multi-disciplinary collaborative approach be initiated to achieve the objective of developing high yielding, disease resistant varieties with better quality. Breeding approaches must be refined to make the best use of existing variability. Search for cytoplasmic genetic type of male sterility or temperature sensitive male sterility and development of inbreds be started as an effort targeted towards exploitation of heterosis.

Table 7
Cumin varities released for cultivation in India.

S.NO.	Name of variety	Salient characteristics
1	RZ-19	It is developed through recurrent single plant progeny selection from collection of Kekri (Ajmer).The plants are erect with pink flowers and bold grey pubescent seeds. As compared to local check the variety is more tolerant to wilt as well as blight. It matures in 140 to 150 days and produces an average yield of 5.6 q/ha
2	RZ-209	It is developed through recurrent single plant progeny selection in a local collection from Ahore (Jalore).The variety has shown higher resistance to wilt and blight diseases. It matures in 120 to 130 days and produces an average yield of 6.5 q/ha
3	RZ-223	It is developed through mutation breeding in UC-216. The variety has shown higher resistance to wilt and blight diseases. It matures in 120 to 130 days and produces an average yield of 6.0 q/ha.
4	RZ-341	The variety has been developed through polycross between high volatile oil content vs. low volatile oil content. The plants are bushy and semi-erect with long and bold seeds, lesser infestation of wilt, blight and powdery mildew and also have high volatile oil contents is 3.87%. It matures in 120-130 days and produces an average yield of 4.50 q/ha.
5	RZ-345	The variety has been developed through recurrent selection based on individual plant progeny (half sib) performance in accession 345. The plants are bushy and semi-erect with long and bold seeds,lesser infestation of wilt, blight and powdery mildew and also have high volatile oil contents is 3.83%. It matures in 120-130 days and produces an average yield of 6.07 q/ha.
6	Guj. Cumin-1	Yield potential (5.41q/ha).
7	Guj. Cumin-2	Yield potential (6.22q/ha), better grain quality
8	Guj. Cumin-3	Yield potential (6.20q/ha), high oil content and resistant to wilt.
9	Guj. Cumin-4	Yield potential (12.50q/ha). *Fusarium* wilt resistant variety with bold non splitting seeds and have high oil content of 4.2%

11. CONCLUSION

Fruits of *Cuminum cyminum commonly* consumed as condiment across the world. Assessment of genetic polymorphism using modern biotechnological equipment such as molecular markers are the vital part of plant breeding because its use helps the plant breeder to develop new varieties. Knowledge of genetic relationship is essential in several crop breeding programs e.g. improvement of cultivars, management of germplasm and evolution of conservation strategies. Molecular characterization of the genotypes gives specific information about the extent of genetic polymorphism which helps in the development of a suitable breeding program. Therefore, there are certain possibilities that in future these biotechnological tools especially molecular markers will become the most important contrivance to enhance the productivity and characterize them for various genetic improvement programmes.

REFRENCES

1. Ahmad Reza Gohari and Soodabeh Saeidnia) Phytochemistry of *Cuminum cyminum* seeds and its Standards from Field to Market. Pharmacognosy Journal,2011; 3(25):1-5c o g J .P H c o g J
2. Alireza Bahraminejad,Ghasem Mohammadi-Nejad,Mihdzar Abdul Khadir.Genetic diversity evaluation of Cumin (*Cumin cyminum* L.) based on phenotypic Characteristics. AJCS, 2011,5(3):304-310
3. Alireza Bahraminejad,Ghasem Mohammadi-Nejad, Mihdzar Abdul Kadir, Mohd Rafii Bin Yusop Molecular diversity of Cumin (*Cuminum cyminum* L.) using RAPD markers. AJCS , 2012; 6(2):194-199
4. Allaghadri T, Rasooli I, Owlia P, Nadooshan MJ, Ghanfari T, Taghizadeh M, et al. Antimicrobial property, antioxidant capacity, and cytotoxicity of essential oil from cumin produced in Iran. J Food Sci. 2010;75:H ; 54-61.
5. Amin Baghizadeh, Mahmood Salar Karimi, Shahram Pourseyedi.Genetic diversity assessment of Iranian green cumin genotypes by RAPD molecular markers.Intl. J. Agron. Plant. Prod.2013; Vol., 4 (3): 472
6. Azza A. Tawfik,G. Noga.(2001).Adventitious shoot proliferation from hypocotyl and internodal stem explants of cumin. Plant Cell, Tissue and Organ Culture ,1992 66(2) :141-147
7. Azza A. Tawfik, G. Noga. Cumin regeneration from seedling derived embryogenic callus in response to amended kinetin Plant Cell.Tissue and Organ Culture April 2002, 2002;Volume 69(1): 35- 40
8. Bukhari SB, Iqbal S, Bhangar MI. Antioxidant potential of commercially available cumin *(Cuminum cyminum Linn*.) Int J Food Sci Nutr. 2009; 60:240–7.
9. Bettaieb I, Bourgou S, Wannes WA, Hamrouni I, Limam F, Marzouk B. Essential oils, phenolics d antioxidant activities of different parts of cumin (*Cuminum cyminum* L.) J Agric Food Chem 2010; 58:10410–8.
10. Daljeet Kaur, Ramica Sharma. An Update on Pharmacological Properties of Cumin.International Jounal of Research in Pharmacy and Science. 2012; 2(4):17-22
11. Derakhshan S, Sattari M, Bigdeli M. Effect of cumin (*Cuminum cyminum* L.) seed essential oil on biofilm formation and plasmid integrity by *Klebsiella pneumoniae*. Pharmacog Mag, 2010; 6:57-61.
12. Dorman HJD, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J Appl Microbiol; 2000; 88:308-16.
13. Derakhshan S, Sattari M, Bigdeli M. Effect of sub-inhibitory concentrations of cumin (*Cuminum cyminum* L.) seed essential oil and alcoholic extract on the morphology, capsule expression and urease activity of *Klebsiella pneumoniae*. Int J Antimicrob Agents. 2008; 32:43
14. Esmaeil Ebrahimie, A.A. Habashi et al. A rapid and efficient method for regeneration of plantlets from embryo explants of cumin (*Cuminum cyminum*). Plant Cell, Tissue and Organ Culture October, 2003;75,(1):19-25

15. Evans WC. Trease and Evans Pharmacognosy. 14 ed. London WB Saunders Company Ltd, 1997; p. 267-8.
16. Fosket D E.Hormonal control of morphogenesis in cultured tissues in F Skoog (ed) .plant growth substances. Springer- Verlag Heidelberg, 1979; pp 362-369.
17. Gagandeep S, Dhanalakshmi S, Mendiz E, Rao AR, Kale RK. Chemopreventive effects of *Cuminum cyminum* in chemically induced forestomach and uterine cervix tumors in murine model systems. Nutr Cancer, 2003; 47:171–80.
18. Hossein Rostami-Ahmadvandi , Kianoosh Cheghamirza , Danial Kahrizi , Sohbat Bahraminejad. Comparison of morpho-agronomic traits versus RAPD and ISSR markers in order to evaluate genetic diversity among *Cuminum cyminum* L. Accessions. AJCS, 2013;7(3): 361-367.
19. Haghparast A, Shams J, Khatibi A, Alizaseh AM, Kamalinejad M.Effects of the fruit essential oil of *Cuminum cyminum* Linn. (Apiaceae) on acquisition and expression of morphine tolerance and dependence in mice. Neurosci Lett, 2008; 440:134–9.
20. Johri RK. *Cuminum cyminum* and *Carum carvi.* An update. Phcog Rev, 2011; 5:63-72.
21. Jakher M L and Choudhary M R. Journal of plant science Research, 2012; 28 (2) 203- 209.
22. Jakher M L Sastry E V D Dhyal L S and Rathore V S. Indian Journal of plant physiology (special issue) 2004;pp 685-688.
23. Jakhar M L,Ganga Ram and Babu Lal.The Journal of plant Science Research. 2013; 29(2) pp 255-263.
24. Koppula S, Kopalli SR, Sreemantula S. Adaptogenic and nootropic activities of aqueous extracts of *Carum Carvi* Linn (Caraway) fruit: an experimental study in wistar rats. Aust J Med Herb, 2009; 21:76-9.
25. Kitajima J, Ishikawa T, Fujimatu E, Kondho K, Takayanagi T. Glycosides of 2-C-methyl-D-erythritol from the fruits of anise, coriander and cumin. Phytochemistry, 2003; 62:115-20.
26. Kafi M. Cumin (*Cuminum cyminum*) Production and Processing. Mashhad: Ferdowsi University of Mashhad press, 2002.
27. Khatibi A, Hagrarast A, Shams J, Dianati E, Komaki A, Kamalinejad M . Effects of the fruit essential oil of Cuminum cyminum L.on the acquisition and expression of morphine-induced conditioned place preference in mice. Neurosci Lett, 2008; 448:94–8.
28. Manish Chittora and Kartikeya Tiwari. BIOLOGY AND BIOTECHNOLOGY OF CUMIN. International Journal of Bioassays ISSN: 2278-778X 02(07), 2013; 1066-1068.
29. M.l. Jakhar and S S Rajput.Present status and Strategies for improvement in seed spices.crop improvement an integrated Approach.MD publications Pvt Ltd New Dehli, 2013; 239-262.
30. Mahesh CM, Gowda KPS, Gupta AK. Protective action of *Cuminum cyminum* against gentamicin- induced nephrotoxicity. J Pharmacy Res, 2010; 3:753-7.
31. Najda A, Dyduch J, Brzozowski N. Flavonoid content and antioxidant activity of caraway roots (Carum carvi L.) Veg Crops Res Bull, 2008; 68:127–33.
32. Nalini N, Sabitha K, Vishwanathan P, Menon VP. Influence of spices on the bacterial (enzyme) activity in experimental colon cancer. J Ethnopharmacol, 1998; 62:15–24.
33. Shipla K S Kumar V D and Sujatha M.Plant cell tiss organ culture, 2010; 103 387-401.
34. Shukla M R Subash N Patel D and Patel S A.In vitro studies in cumin (*cumiinum cyminum* L).In Edision S Ramana K V Sasikumar B Nirmal Babu K and Santosh J Eapen(Eds) Biotechnology of Spices Medicinal and Aromatic plants Calicut Indian Society for Spices Calicut Kerala Indian, 1997; pp 45-48.
35. Takayanagi T, Ishikawa T, Kitajima J. Sesquiterpene lactone glucosides and alkyl glycosides from the fruit of cumin. Phytochemistry, 2003; 63:479-84.
36. Thippeswamy NB, Naidu A.Antioxidant potency of cumin varieties - cumin, black cumin and bitter cumin - on antioxidant systems. Eur Food Res Technol, 2005; 220:472–6.
37. Willatgamuva SA, Platel K, Sarawathi G, Srinivasan K Antidiabetic influence of dietary cumin seeds *(Cuminum cyminum*) in streptozotocin induced diabetic rats. Nutr Res, 1998. 18:131–42.