stress-free area for commercial cultivation of any crop. Plant breeding strategies for dealing with diseases and insect pests, two of the very important biotic stresses, have been dealt with in Chapters 27 and 28. Abiotic stresses result due to moisture, temperature (high/low), minerals (deficiency/toxicity), salinity, soil pH, air pollution, etc. In Chapters 24 to 26, we consider the meanings of, and the plant breeders' answer to the most important abiotic stresses.

24.2. IMPORTANCE OF ABIOTIC STRESSES

Abiotic stresses not only determine the geographical and regional (even subregional) distributions of crops, but they also dictate if a potentially arable (= cultivable) land piece can actually be used for cultivation. According to an estimate, 24.2% of the world's geographical area is potentially arable. But only 10.6% of the geographical area is under actual cultivation, and the rest of the area is not accessible for cultivation due to one or more abiotic stresses (Table 24.1). Even in India, about 13.6% of the geographical area is potentially, but not actually, arable. Temperature is the most effective factor affecting crop distribution; moisture and soil-chemical properties are also important factors.

TABLE 24.1

Per cent of geographical area potentially and actually arable

Region	*Arable area as per cent of geographical area*	
	Potential	*Actual*
World	24.2	10.6
Asia	24.1	18.9
India	56.0	43.6

Abiotic stresses are the primary sources of yield losses; they account for Ca. 71% of total reductions in crop yields. It is estimated that only 10% of the world's arable area is not subject to an abiotic stress. Drought is the main abiotic factor as it affects 26% of the arable area. Mineral toxicities/deficiencies are second in inportance, while freezing stands close third. (Table 24.2). It may be pointed out that the relative importance of different abiotic stresses varies greatly depending on the geographical region and the specific location within a given region. In addition, quantification of the degree of an abiotic stress is generally difficult and is often influenced by other factors of the environment. For example, the degree of moisture stress experienced by a crop will be greater under warmer conditions than that under

TABLE 24.2

The fraction of world's arable land subject to an abiotic stress

Abiotic stress	*Fraction (%) of arable land*
Drought	26
Mineral (toxicity/deficiency)	20
Freezing	15
NO STRESS	10

(Dudal, 1976; quoted in Blum, A., 1988)

relatively cooler environments, the soil moisture level and air humidities being comparable under both the environments. In addition, the severity of the effects of a stress on a crop depends greatly on the duration of stress and on the stage of crop growth at which the stress has occurred.

24.3. CHARACTERISTICS OF ABIOTIC STRESSES

1. The characteristics of an abiotic stress may vary considerably depending on the location.
2. The relative importance of different abiotic stresses is mainly region/location-specific.
3. The occurrence and the degree of some of the stresses are unpredictable, *e.g.*, drought, while those of some others are reliably known for a given location.
4. The degree of some stresses is likely to vary during the crop season.
5. Some stresses can be relieved by appropriate management practices, *e.g.*, drought, salinity etc., while some others are virtually impossible to manage, *e.g.*, temperature stress.
6. A given abiotic factor may increase/decrease the level of another abiotic stress, *e.g.*, in a saline soil, moisture stress would enhance salinity stress.
7. Different plant/crop species show marked differences in their abilities to withstand a given stress.
8. Different varieties of a crop also show large differences in their ability to tolerate/resist abiotic stresses.
9. Different growth stages of crops may show marked differences in their tolerance to an abiotic stress. For example, maize shows good tolerance to alkalinity at germination stage, but at established stage it exhibits only poor tolerance.
10. Stress during the reproductive phase of crop causes far more economic loss than comparable stress during the earlier phases. In some cases, adverse effects of a stress during an earlier phase may be almost entirely compensated, *e.g.*, moisture stress during the vegetative phase of sorghum (Table 24.3).
11. The effects generated by one abiotic stress may overlap some of those generated by another stress. For example, salinity stress generates some features produced by drought stress, so that strains developed for salt resistance also show enhanced drought tolerance.

It should, therefore, be clearly recognized that the following discussion on abiotic stresses is in very general terms. A specific and pecise discussion is virtually impossible, except for specified situations, which are of little relevance for a text book.

24.4. MINIMISING LOSSES DUE TO ABIOTIC STRESSES

Abiotic stresses may lead to suboptimal yields to total crop failure. These losses can be reduced/eliminated by the following approaches: (1) crop management, and (2) development of resistant varieties.

TABLE 24.4

Performance of open-pollinated (during 1928 – 1936) and hybrid varieties (during 1972 – 1976) of maize the same seven states of USA. The periods sampled included both good and bad years. Increased yield potential of hybrids seems to improve their performance in stress environments (Harvey, 1977; quoted in Lewis and Christiansen, 1982)

Type of variety	*Period under study*	*Yield (Q/ha)*		*Decline (%) in the bad years*
		Good years	*Bad years*	
Open-pollinated	1928 – 1936	15	5	63
Hybrid	1972 – 1976	57	40	30

survival under stress may not involve the same mechanisms that lead to higher productivity under stress.

2. *Selection for Yield.* In general terms, ***resistance to an abiotic stress*** may be defined as the ability of a plant/line to produce higher economic yields than other plants/lines subjected to the same/comparable levels of the given stress. This is what a farmer would ultimately expect from a resistant cultivar. But yield may not be the best criterion for selection in the development of resistant cultivars particularly at the stage of selection of parents, since it could lead to the rejection of potentially valuable germplasm/sources of resistance. It has been suggested that selection should initially be based on high biomass yields under the given stress. The harvest index of the selected material could then be improved to evolve a high yielding variety; it is assumed here that harvest index and stress resistance are independent of each other. An example is provided by the initially developed verticillium wilt resistant varieties of cotton (*G. hirsutum*); these varieties produced large biomass but few bolls. Subsequently, high yielding wilt resistant varieties were bred from these materials.

3. *Selection for Traits Contributing to Stress Resistance.* The greatest progress may be expected if selection is based on some trait that contributes to stress resistance. For such an approach to be successful, it should be established that the trait in fact contributes to resistance to the given stress and that it is associated with high yields. Many traits have been implicated in resistance to abiotic stresses, but there are few good examples that could serve as good selection criteria.

24.5. BREEDING FOR DROUGHT RESISTANCE

Drought seems to be rather difficult to define and more difficult to quantify. For example, the common criteria used in the various definitions are precipitation, air temperature, relative humidity, evaporation from a free water surface, transpiration from plants, wind, airflow, soil moisture and plant conditions. A working definition of ***drought*** may be "the inadequacy of water availability, including precipitation and soil moisture storage capacity, in quantity and distribution during the life cycle of a crop to restrict (the) expression of its full genetic yield potential" (Sinha, 1986). Therefore, under conditions of drought, water stress develops in the plants as the demand exceeds supply of water; this may occur due to atmospheric or soil conditions, and is reflected in a gradient of water potentials developed between the soil/soil-root interface and lea t. ng organ. Thus *moisture stress* may be defin d as the inability of plants to t otranspirational demand. Moisture stress is ikely to develop to a different r d nt plant organs along this gradient (Blum, 1988).

24.6. EFFECTS OF D GHT ON PLANT GROWTH AND DEVELOPMENT

Water stress has marked ct on cellular processes, plant growth, development and economic, yield. At the ce ar lev l, it affects the following structures/processes: (1) structures of membranes an ganelle , (2) hydration and structures of macromolecules like proteins and nucleic aci nd (3) p essure differential across the membrane-cell wall complex, which in turn af cell expa sion.

Water stress is usual asured as leaf-water potential since leaves are directly involved with the production of a ates for growth and yield. As water potential declines, pressure or turgor potential also d es; the decline in turgor potential is much more when there is no osmoregulation in resp water stress. *Osmoregulation* or *osmotic adjustment* refers to the active accumulatio solutes in the cells during the period in which water stress develops. The solutes a ulated by different plants are considerably different: they range from photosynthetic pro like sugars, fructans, etc., through organic acids of which amino acids are the most not to inorganic ions of which K^+ is the most important. *Osmotic adjustment is finite, an fficient time is required for solute accumulation.* Generally, the relationships between c ar effects of water stress and those observed at plant level are not clearly understood. It en much less clear whether these effects are mediated by water potential or turgor pot l. However, *performance of a crop under water stress will be affected by the integrat fects of water stress at all the levels of plant organization.*

An oversimplified ription of the development of water stress in a field and its effects on the crop is as follow itially, a moderate level of stress develops at which photosynthesis continues but leaf e sion ceases; a part of the photosynthate is used for osmotic adjustment or it may b titioned to other organs, *e.g.*, roots (Table 24.5). Thus plant growth is inhibited by mod levels of stress that reduce cell enlargement. This increases root/shoot ratio, preve increase in leaf area and, thereby, reduces the rate of water stress development. Osmot justment would occur; this will protect the cells from extreme desiccation and allow ntinued gas exchange.

As water stress i ses, older leaves senesce to various degrees; this reduces the leaf area of plants. As a re water use by the crop may decline in proportion to the reduction in leaf area and increas hydraulic resistance in the plants. As long as bulk leaf turgor is maintained in the liv ves due to osmotic adjustment and/or reduced evapotranspiration, stomata may remain n and photosynthesis may continue. The rate of photosynthesis during this phase dep partly on stomatal resistance and partly on mesophyll resistance to CO_2 diffusion.

If a moderate le f water deficit develops in the meristems, differentiation of organs contributing to yiel ll be directly and adversely affected. Fortunately, reproductive meristems are locate ites removed from the sites of greatest moisture stress in plants, and they have a greater ca ty for osmotic adjustment. As a result, *the level of water stress in*

TABLE 24.5

Source-sink relationships during different growth phases and moisture stress regimes

Growth phase/ stress regime	*Major sink*	*Sink serves as*	*Source*	*Remarks*
Vegetative	Roots	Source of cytokinins	Developed leaves	Developing shoot also serves as sink so long as photosynthates accumulate
Water stress	Roots		Developed leaves	
Water stress relieved	Reviving shoot		Roots	
Reproductive phase	Developing grain	Triggers ABA synthesis	Flag leaf, panicle	

reproductive meristems is lower than that in the transpiring leaves of a plant at any given time.

If water stress continues to increase, turgor is lost, stomata close fully, growth ceases, remaining live leaves (of cereals) roll up, gas exchange drops to zero, carbon is lost by respiration, tissue water continues to decrease slowly, and the plant enters the prelethal, nonreproductive stage of survival. In the absence of transpiration, temperature of the remaining leaves increases to lethal levels, and all leaves die. But meristems may still survive; once the meristems die, a plant is considered as dead.

The various plant processes are affected at different levels of water stress. For example, cell expansion is the first to be affected, cell division followed by photosynthesis are moderately sensitive, while photosynthate translocation is probably the most resistant process. In addition, the various processes exhibit a detectable response to water stress after considerably different time periods after the onset of stress (Table 24.6). For example, water potential, turgor pressure and stomatal resistance are affected within seconds/minutes, leaf senescence occurs after days and effects on yield are observable only after days to months.

TABLE 24.6

Time-scale of some plant processes, which may influence their drought tolerance

Time-scale	*Phenomenon*
Minutes or less	Turnover of some proteins; stomatal movement
Hours	Production of heat shock proteins or dehydrins, leaf movement, wilting, osmotic adjustment, response to ABA
1-2 days	Cellular hardening—induction of house-keeping genes, seed set, floral initiation, flowering
Several days to weeks	Canopy development, leaf senescence, root system development
Weeks to months	Clock controlling development, *e.g.*, vernalization, time to flowering, grain filling

24.7. TYPES OF DROUGHT ENVIRONMENT

The breeding methodology as well as the resistance mechanism that should be developed will depend, to a large extent, on the type of drought environment to which the crop will be subjected to. In general, the following three types of environments can be associated with drought: (1) stored moisture environment, (2) variable moisture environment and (3) optimal moisture environment. It may, however, be pointed out that numerous combinations of these environments occur in reality.

24.7.1. Stored Moisture Environment

In this type of environment, the crop completes its life cycle on the moisture stored in the soil during a prior wet or rainy season. As a result, the level of moisture stress will depend on the amount of moisture stored in the soil, the duration of the crop and the rate of evapotranspiration. In such environments, crops become subjected to moisture stress during their terminal phase of growth and development. The likelihood of success of breeding for drought resistance is rather high, and a spectrum of traits can be exploited for this purpose.

24.7.2. Variable Moisture Environment

This type of drought environment is characterised by alternate dry and wet periods of varying lengths. Plants grown in such environments must be able to take advantage of the periodic rainfall and also to survive, with minimum detrimental effects, the periods of water stress. The periodic and variable nature of water stress is likely to reduce the chances of success of breeding programmes for drought resistance.

24.7.3. Optimal Moisture Environment

The crop is grown with adequate moisture during most of its life cycle; drought occurs occasionally at highly unpredictable stages of growth and development. The period of drought may be limited to a part of one day when evapotranspiration greatly exceeds root uptake. But ordinarily it is associated with a period of less than normal precipitation. The effects of drought in such environments are likely to be rather severe in view of the inadequate time available for the plants to become adjusted to water stress. Breeding for drought resistance for such environments would be extremely difficult.

24.8. DROUGHT RESISTANCE

Drought resistance may be defined as the mechanism(s) causing minimum loss of yield in a drought environment relative to the maximum yield in a constraint-free, *i.e.*, optimal, environment for the crop. However, it does not exist as a unique heritable plant attribute. The various mechanisms by which a crop can minimise the loss in yield due to drought are grouped into the following three categories: (1) drought escape, (2) dehydration avoidance and (3) dehydration tolerance; these are briefly discussed below (Table 24.7).

24.8.1. Drought Escape

Drought escape describes the situation where an otherwise drought susceptible variety performs well in a drought environment simply by avoiding the period of drought. *Early maturity is an important attribute of drought escape, and is suitable for environments subjected to late-season drought stress.* Early varieties generally have lower leaf area index, lower total evapotranspiration and lower yield potential. Therefore, this attribute is not suitable for variable moisture and optimal moisture drought environments. An early line of sorghum (jowar) exhibited markedly higher root-length density per unit leaf area than a late isogenic line. This has been interpreted as ability of the early line to maintain a higher leaf

TABLE 24.7

A summary of the various mechanisms of drought escape and drought resistance and their use as selection criteria

Characteristic	*Measurement*	*Association with yield under stress*	*Usefulness as selection criterion*
Drought Escape			
Earliness	Easy	Positive	Quite useful
Dehydration Avoidance			
1. *Reduced Transpiration ('water saver' plants)*			
Stomatal sensitivity	Difficult	Positive (?)	May be useful
Osmotic adjustment	—	Positive	Useful
Cuticular wax	Easy	Positive (?)	Possibly useful
Abscisic acid	Difficult	Positive (?)	Doubtful
Leaf pubescence	Easy	Positive (?)	Questionable
Leaf angle and leaf movement	Easy	—	—
*Leaf rolling	Visual score (0-5); easy	Negative	Quite useful in rice
2. *Increased Water Uptake ('water spenders')*			
Root characteristics	Very difficult	Variable	Limited
a. Deep root system	Very difficult	Positive (?)	Useful**
b. Root-length density	Very difficult	Doubtful	Useful†
c. Root hydraulic resistance	—	Positive (?)	Doubtful
Dehydration Tolerance			
†† Maintenance of membrane integrity	Easy	Doubtful	Doubtful
†† Seedling survival	Easy	Doubtful	Useful for seedling drought tolerance
†† Seedling rooting pattern	Easy	Doubtful	Doubtful
†† Seed germination	Easy	Nil	Nil
Translocation of stem reserves	Easy	Positive	Useful
Proline accumulation	Difficult	Positive	Useful (?)

* Selection for delayed leaf rolling.
** When deeper soil layers have water reserve.
† No water reserve in deeper soil layers.
†† Under water stress conditions.

water potential at a given soil-moisture potential than its late isoline. *It appears from this and many other studies that maturity duration may be more deeply involved in the plant-water relations than its mere effects on total evapotranspiration.*

24.8.2. Dehydration Avoidance

Dehydration avoidance is the ability of a plant "to retain a relatively higher level of 'hydration' under conditions of soil or atmospheric water stress." This results in the various physiological, biochemical and metabolic processes of plants that are involved in growth and yield not being internally exposed to stress and, thereby, they are protected from water stress (Blum, 1988). The common measure of dehydration avoidance is the tissue water status as expressed by water or turgor potential under conditions of water stress. This can be achieved either by reducing transpiration (such plants are often called ***water savers***) or increased water uptake (such plants are often termed as ***water spenders***). Wild species are readily classifiable as 'water savers' and 'water spenders', but crop plants ordinarily exhibit a combination of both features, probably as a result of selection by man.

24.8.2.1. Reduced Transpiration. Water saving mechanism is common in xerophytes, which have evolved for survival under extreme water stress conditions; ordinarily, they show poor biomass production. Water saving species reduce transpiration mostly by closure of their stomata in response to water deficit well before wilting (***stomatal sensitivity to water stress***). Stomata are responsible for the bulk of transpiration, and also for gas exchange in respiration and photosynthesis. Therefore, stomatal closure is likely to interfere with photosynthesis, and drought resistance mechanisms based on stomatal sensitivity and reduced transpiration are generally opposed to the maintenance of a higher yield potential. In water stressed plants, stomata may remain open during the early morning hours, and close as solar radiation increases. An extreme example is provided by the crassulacean acid metabolism (CAM) in some succulents, where stomata close during the day and open during the night, when CO_2 is fixed by them. Stomatal activity *per se* is an imperfect criterion of drought resistance, unless it is related to both carbon fixation and transpiration under stress.

24.8.2.2. Osmotic Adjustment. It is an important mechanism of dehydration avoidance. Osmoregulation is positively associated with yield under stress conditions, as it allows growth and results in delayed leaf death by maintaining turgor pressure and possibly, some other unknown mechanisms. But this mechanism of dehydration avoidance may reduce photosynthesis upon recovery and could lower potential yields if it is associated with smaller cell size (which may reduce both source and sink sizes). Critical and elaborate studies have shown that the daily net gain in carbon in stressed plants was always positive due to osmoregulation, and the carbon compounds stored for osmoregulation were immediately available for biomass production when the stress was relieved. In conclusion, *osmotic adjustment positively affects growth and yield under stress. It is probably one of the most important and effective components of drought resistance.*

The role of different mechanisms may change with the stage of plant development. For example in sorghum, stomatal sensitivity to water stress seems to be the main mechanism during vegetative phase, while after flowering osmoregulation and turgor maintenance were important.

24.8.2.3. Abscisic Acid (ABA). ABA is known as *'stress hormone'* as its concentration increases in response to stresses, including water stress. Water deficit is sensed by roots, which begin to synthesize ABA within 1 hour (*e.g.*, in maize) of the onset of water stress. ABA is transported via xylem from roots to leaves within minutes to hours, its half-life in leaf being ~30 min. Xylem ABA concentration decreases sharply and stomata open in less than one day after watering of the stressed plants. *ABA plays a major role in water stress avoidance by effecting stomata closure, reduction in leaf expansion and promotion of root growth.* As a result, mutants partly deficient in ABA biosynthesis are more stressed at the cellular level than are normal plants, when both are subjected to the same level of water stress. In some crops, ABA accumulation was positively associated with yield under stress, while in several others the association was not clear.

Expression of several genes is induced by one or more stresses; these genes are called ***stress-responsive genes*** and the proteins they encode are known as ***stress proteins.*** *The stress proteins are involved in a broad range of pathways, but the functions of many such proteins are yet to be discovered.* Such proteins have been observed in response to heat, cold, salinity, drought and other stresses. Some genes are induced ~1 hr after dehydration begins; they are called ***early-responsive to dehydration (ERDS) genes.*** Water stress tolerance appears to involve the accumulation of such compounds that protect the cells from damage at low water potentials, *e.g.*, osmolytes, chaperones, proteinase inhibitors, proton ATPases, and a large set of proteins with protective functions. Many of the stress proteins, *e.g.*, dehydrin, osmotin, Lea proteins, etc., are also produced in response to ABA; genes encoding such proteins are called ***ABA-responsive (ABAR) genes.*** ABAR genes have in their promoters an ***ABA responsive element (ABARE),*** which has ACGT as its core sequence. ABA-induced proteins are mostly hydrophilic and are proposed to function in sequestration of ions, protection of other proteins/membranes, and renaturation of unfolded proteins. However, convincing evidence for these functions are yet to be obtained.

24.8.2.4. Cuticular Wax. Transpiration also occurs through cuticle; the amount of transpiration depends mainly on the wax deposited within and over the cuticle. The genotypic potential for wax deposition is best evaluated in plants subjected to water stress. But the effect of cuticular wax on transpiration is small and, for a given plant, increase in wax load beyond a given threshold would not reduce transpiration. The shape and the angle of wax deposition may affect leaf reflectance within the spectrum range of 400 to 700 nm, which in turn may affect net radiation and leaf temperature. For example, increased glaucousness (due to structural properties of wax) in wheat and sorghum reduced net radiance and leaf temperature, which improved their yields under water stress.

24.8.2.5. Leaf Characteristics. ***Leaf pubescence*** generally increases leaf reflectance and reduces net radiation resulting in lower leaf temperature under high irradiance. This trait shows positive association with yield under stress in some cases but not in others. Net radiation can also be reduced by ***altering the leaf angle*** from 'horizontal', which receives the maximum radiation. Thus in cereals, 'erect leaf' lines perform better than 'lax leaf' lines under moisture stress. The ***paraheliotropic movement of leaves***, particularly in legumes achieves the same end. ***Leaf rolling*** is the most familiar response to water stress, especially in cereals. Leaf rolling is induced by loss of turgor; therefore, it is delayed by osmotic adjustment. Hence *delayed leaf rolling is an indication of turgor maintenance as a component of dehydration avoidance; it is used as an important selection criterion for dehydration avoidance in rice.*

24.8.2.6. Increased Water Uptake. Water uptake depends mainly on the characteristics of root system, which may be described and measured in various ways, *e.g.*, root-length density, root axial resistance, root radial resistance, etc. Some broad generalizations about root system and its possible role in water stress resistance are as follows.

1. When soil moisture is unlimited at deeper soil horizons, a ***deep root system*** is a distinct and effective component of drought resistance. Breeding for drought resistance in upland rice in U.S.A., involved the genetic improvement of root extension and penetration.
2. But when there are no additional moisture reserves at deeper soil layers, large ***root-length density*** and small ***root (hydraulic) resistance*** would contribute to the maintenance of higher water potential.
3. Root distribution pattern is affected by water status of the soil. In situations of transient soil drying and wetting, a dense root system and/or a low root resistance is important in the maintenance of a higher leaf water potential.
4. In stored moisture environments, it is important to minimise evapotranspiration during the early season. This can be achieved either by reducing leaf-area index or by the development of a greater hydraulic resistance in the root. A selection for smaller root xylem vessel diameter resulted in increased root axial hydraulic resistance.

Direct root observation are, however, tedious and costly; therefore, they are of limited utility as selection criteria. A reasonable alternative is to measure their function and efficiency as leaf water potential when the soil is drying.

24.8.3. Dehydration Tolerance

When cells lose turgor and dehydrate, there is (1) reduced chemical activity of water, (2) increased concentration of solutes and macromolecules, (3) removal of water of hydration from macromolecules, and (4) alterations in cellular membranes. ***Dehydration tolerance*** of a genotype means that a significantly lower level of changes are induced in it than those in another genotype when both of them are subjected to the same level of dehydration. The various measurements of dehydration tolerance are briefly considered here.

24.8.3.1. Maintenance of Membrane Integrity. It is usually determined by the leakage of solutes (including various electrolytes, amino acids, saccharides, organic acids, hormones, etc.) from cells. It should be noted that all test plants must be subjected to the same stress history, and its association with drought resistance at plant level remains to be shown.

24.8.3.2. Plant Growth. Plant growth under stress is often used as an index of drought resistance, but it is a function of both dehydration avoidance and dehydration tolerance. Generally it is measured using seedlings by assessing either (1) growth or (2) survival.

1. ***Seeding survival*** or recovery after stress is a useful index of dehydration tolerance, and it may be used as a selection criterion for seedling drought tolerance. It may also reflect the tolerance of adult plants in many species.

2. ***Seedling growth*** under stress serves more or less the same purpose as seedling survival.
3. ***Seed germination under osmotic stress*** created by mannitol or polyethylene glycol (PEG) has little value as a measure of drought tolerance/resistance, at least in a large number of crop species.
4. Stem reserves are a powerful resource for grain filling in stress-affected plants during grain-filling stage. The capacity for ***stem reserve mobilisation/translocation*** appears to be related to drought tolerance/resistance, and could be signalled by the ABA accumulated in response to water stress. If, however, photosynthesis continues during stress, the translocation of stem reserve is reduced.
5. In cereals, spike maintains a better water potential under stress, and provides more assimilates to the developing grain than does the flag leaf. *Awns are far more water-use efficient than flag leaf and even glumes.* Therefore, the ***presence of a large amount of awns*** is a drought-adaptive attribute in cereals. In extreme cases, the spike, including awns, surface area may surpass flag-leaf area.
6. Proline, a cell compatible solute, is accumulated to very high levels during stress; its concentration declines rapidly upon rehydration. Proline also accumulates in response to other stresses like salinity and extreme temperature and contributes to plants tolerance to them. Thus ***proline accumulation*** appears to be involved in tolerance to water and other stresses. Accumulation of very large amounts of proline contributes to osmotic adjustment; it may also serve as cytoplasmic osmotic balance for potassium accumulation as the main osmoticum in the vacuole. It serves as a protectant of various enzymes and biological membranes subjected to desiccation and heat stress. However, it is extremely important that the proline contents of different genotypes/lines are measured when their tissue water potentials are practically the same. This is essential to avoid wrong conclusions as proline accumulates exponentially with the reduction in tissue water potential.

In conclusion, a dependable yardstick to measure dehydration tolerance has not yet been developed. In practice, any one of the above criteria may be used to assess dehydration tolerance.

24.9. GENETICS OF DROUGHT RESISTANCE

Existence of genetic variation for drought resistance has been demonstrated in many crops. Drought resistance was estimated as yield stability (*e.g.*, in wheat, rice, maize, barley, sorghum), leaf water potential (sorghum, wheat, rice, soyabean, cotton), leaf rolling (rice), root growth (sorghum, rice, oat, wheat, maize), root xylem diameter (wheat), osmotic adjustment (wheat, sorghum), stomatal conductance (upland cotton), ABA accumulation (sorghum, rice, wheat), canopy temperature (maize, cotton), seedling establishment and growth (alfalfa), seedling recovery after stress (maize), growth under stress (maize), resistance to flower shedding and sustained pod formation under stress (rajma, *P. vulgaris*), and proline accumulation (barley and *Brassica* sp.).

The genetic control of these traits ranges from oligogenic to polygenic (Table 24.8).

aspect of such manipulations, however, remains that the basal metabolism of the plant should be able to sustain a high rate of accumulation of the concerned osmolytes without too much of a 'cost' to the plants.

24.11. RELATIONSHIP BETWEEN DROUGHT RESISTANCE TRAITS AND YIELD

A trait that is theoretically expected to be associated with drought resistance is investigated systematically to establish this relationship. (1) First suitable techniques for its measurement are developed. (2) Then the germplasm is screened to assess the genetic variability for this trait. (3) Correlations between such traits and yield under stress may be indicative of their value in breeding programmes. (4a) However, this must be confirmed by comparing the performance for near isogenic lines for each individual trait. Comparison of near-isogenic lines, however, requires considerable time and effort and is best suited for oligogenic traits. A more practical approach, therefore, would be to (4b) cross two parents differing for 4-5 most important stress resistance traits. Individual F_3 plant progenies are subjected to divergent selection for the traits under study. The values for F_4 families are regressed on those for F_3 families to obtain an estimate of heritability. Yield testing is finally done in F_5 or F_6 using contrasting environments, *i.e.*, non-stress and stress environments. The results from the study allow identification of both the sources of drought (and other stress) resistance as well as the traits associated with the resistance.

Alternatively (4c) a composite cross may be generated by crossing several lines having contrasting expressions for a putative drought resistance trait. The cross is maintained by selfing till it is nearly homozygous; random plants are selected and their progenies evaluated under water stress. Heritabilitis of the drought (or any other stress) resistance trait and yield, and the correlation between them is estimated. If the resistance trait shows moderate to high correlation with yield, and a higher heritability than yield *per se*, it will be a useful selection criterion in breeding for drought resistance.

24.12. SELECTION CRITERIA

A good selection criterion should have the following attributes: (1) it should be easy to estimate/score, (2) it should have high (or at least moderate) heritability, (3) a large genetic variability should exist for the trait, (4) it should exhibit a significant association with drought (or the desired stress) resistance, and (5) it should show a positive association (or at least no association) with yield under stress.

A major factor that has prevented progress for improving yield in water-limited environments is the lack of knowledge of the critical taits that should be selected for achieving the goal. The various selection criteria used in breeding for drought resistance in different crops are briefly outlined below (and summarised in Table 24.8) under the following heads: (*i*) dehydration avoidance, and (*ii*) dehydration tolerance.

24.12.1. Dehydration Avoidance

1. ***Leaf rolling*** is scored visually from 0 (no rolling) to 5 (tightly rolled) in rice either in the morning or at mid-day. It is extensively used as selection criterion as selection criterion at

IRRI, Philippines. It is likely that leaf rolling will predict leaf-water potential in species of low osmotic adjustment, *e.g.*, rice, while in those with greater osmotic adjustment, *e.g.*, wheat and sorghum, it would predict turgor maintenance by osmotic adjustment. In addition, care should be taken to avoid the confusion that may arise due to changes in leaf rolling symptom due to leaf age and plant growth stage.

2. ***A combination of leaf rolling and leaf firing*** is being effectively used in maize and sorghum. ***Leaf firing*** is the drying of leaves due to insufficient transpirational cooling. In maize leaf senescence under stress was significantly negatively correlated (–0.48**) with yield under stress.

3. ***Canopy temperature*** is readily measured with infrared thermometer. In maize it was negatively (–0.56** to –0.73**) correlated with yield under stress and in rice it was negatively correlated ($r = -0.79^{**}$) with spikelet fertility. A sufficient level of water stress is a major prerequisite for applying the method to selection work. The crop must be fairly dense and free of skips, measurements should be made at or immediately after solar noon, and windy conditions should be avoided. Measurements should be repeated 2-3 times a week as the stress-progresses; it should be done during the vegetative phase before the onset of flowering. This selection criterion is being used in breeding programmes of wheat (at ICARDA, Syria), maize and others.

4. ***Leaf attributes*** like dense pubescence, heavy glaucousness, etc. are scored visually. Epicuticular wax load can also be measured relatively rapidly (50–200 samples/day). But these traits are unreliable indicators of drought resistance; it is desirable that they be incorporated into an integrated selection index with a greater weight given to the visual symptoms of wilting/leaf rolling/canopy temperature.

5. ***Leaf water retention*** may be useful in some materials, and its usefulness as the sole criterion of selection is open to question. It should be used as a component of an integrated selection index. It is estimated by sampling turgid leaves, normally early in the morning and then determining their weight after a given period of time (typically, 6 hr) under standard drying conditions. Per cent water retention is calculated from initial and final weights. A higher water retention is the positive response. It is too laborious for practical use if performed manually.

6. ***Root characteristics*** are very difficult to evaluate *en masse*. In any case, root attributes should be reflected in canopy response to stress, which are far easier to evaluate

24.12.2. Dehydration Tolerance

1. ***Seedling growth under PEG stress*** (grown in a standard nutrient solution enriched with PEG) is a useful selection criterion. In alfalfa, a successful selection programme for drought resistance had delayed wilting in a PEG-containing solution as one of the components. Depending on the species, the extent of genetic variation and the PEG concentration, a visual raking of the response to PEG may be sufficient for selection. It is important that the seedlings must not suffer root damage on transfer to the nutrient solution, and PEG may be applied in an step-wise fashion to avoid osmotic shock.

2. ***Growth under stress in the field*** may be used as a selection index provided the measure is sufficiently simple and rapid.

3. ***Plant phenology*** may be used as an index of stress tolerance as drought stress delays or accelerates flowering depending on the growth stage at which stress occurs and on stress intensity. Delayed heading under stress has been used as a selection index in rice. The time interval between pollen shedding and silking in maize under drought stress is shorter in selections with higher yield under stress, and is used as a selection index. Any apparent symptom of sterility under drought stress can be effectively used as a selection index in any crop provided the data are normalised for genotypic variations in flowering date.

4. ***Grain filling by translocated stem reserve*** is an important attribute of resistance of cereals to drought after anthesis. In wheat, it may be estimated as follows. Plants are sprayed to complete wetting by a solution of magnesium/sodium chlorate (4% active ingredient) at 14 days after anthesis. All the plant surface is bleached without killing the plant. Grain filling now proceeds only due to the translocated plant reserves. The 1000-kernel weight of the treated plots is compared with that of the corresponding untreated plots. A smaller difference between the treated and untreated plots indicates a greater reserve translocation. This approach is used experimentally in Israel and Australia.

5. ***Cellular membrane stability under stress*** can be estimated readily by subjecting leaf discs or segments to PEG-induced water stress *in vitro,* and then the leakage of solutes into deionized water is measured conductometrically. But it is not known if this attribute is related to plant performance under drought.

6. In case of wheat, $^{13}C/^{12}C$ ratio appears to be related closely to ***water use efficiency (WUE).*** But its measurement requires expensive instruments, and its value as a selection criterion is yet to be demonstrated.

7. ***Seed germination in an osmoticum*** is meaningless as an indicator of performance under stress.

8. Cell lines have been selected for ***resistance to osmotic stress created by PEG.*** Resistant cell lines usually show osmotic adjustment largely due to accumulation of reducing sugars, potassium, or proline. However, resistance was usually lost upon passage of the cells on nonstress medium. In any case, drought resistant plants are yet to be regenerated from such cell lines.

In conclusion, none of the drought resistance attributes can serve as a reliable index for selection. A successful programme should integrate several criteria into a single selection index. For example, successful recurrent selection within *Tuxepeno* maize used an index based on canopy temperature (major component), growth under stress, leaf firing and synchronous flowering. Similarly, wheat breeders in Israel use an index consisting of canopy temperature, chemical desiccation and the presence of large awns. This programme has produced a widely accepted dryland wheat variety 'Bethlehem'; this variety shows better osmotic adjustment, larger amount of awns, very glaucous leaves, and early maturity.

24.13. CREATION OF DROUGHT ENVIRONMENT

Selection for drought resistance based on any selection index requires a rigorous control over the stress environment. This involves control over not only the water regime, but on other variables like site homogeneity, mineral nutrition, weeds, pests and diseases. The various approaches for the creation of drought environment are briefly summarised below.

1. *Greenhouse environment* can be more precisely controlled than field environment. But care should be taken to consider the commonly occurring gradients in temperature and radiation within the greenhouses.
2. However, *field environment* is highly desirable for selection work. A greater control on moisture stress can be exercised if the breeding site is located in an area of low rainfall (say, < 100 mm) and moisture stress is regulated by supplemented irrigation. Often such a site may lie outside the growing area of the concerned crop.
3. A *"line-source gradient"* can be devised in one of the following three ways.
   (*i*) In case of *sprinkler irrigation*, the test materials are planted in long rows perpendicular to the line of the sprinklers (Fig. 24.2). The accessions may be replicated on the same side or on the opposite sides of the line of sprinklers. Each accession is subjected to a gradient of water stress that increases with the distance from the line of sprinklers.
   (*ii*) A similar gradient develops when water is received by *irrigation in furrows,* since water infiltration decreases with the distance from the irrigation channel. In this case, the line of sprinklers in Fig. 24.2 represents the irrigation channel, other things remaining the same.
   (*iii*) Planting the materials on a *topographical slope,* above an underground water table, creates a similar gradient.

   The 'line-source gradient' system permits the evaluation of materials under a variety of water stress situations in a single experiment.
4. The selected materials should be evaluated under both stress and nonstress conditions. An alternative to line-source gradient approach is *to create two different sets of plots,* one with and the other without water stress, at the same site by providing suitable irrigation regimes.

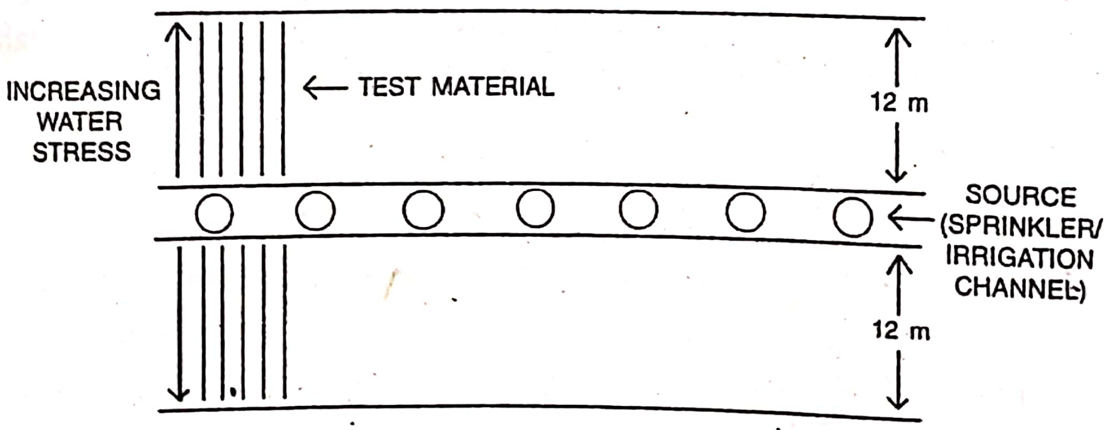

Fig. 24.2. Line-source gradient approach to selection for drought resistance.

24.14. BREEDING METHODS AND APPROACHES

It is important that drought resistance be incorporated into materials with high genetic potential for yield. Yield and yield components are best evaluated under nonstress/optimal environments, while drought resistance must be evaluated under water-stress. Thus selection for stress response needs to be integrated with selection and testing for potential yield under nonstress conditions. The various approaches used for breeding for drought resistance are briefly outlined below.

24.14.1. Adaptation to A Specific Environment

In this approach, varieties are developed specifically for adaptation to moisture deficit environments, and selection and evaluation are carried out under moisture stress. This approach should be the most useful in an environment where crop is grown on moisture stored during the previous season. But a variety developed through this approach may not be able to take advantage of the above normal precipitation in favourable years. In addition, it

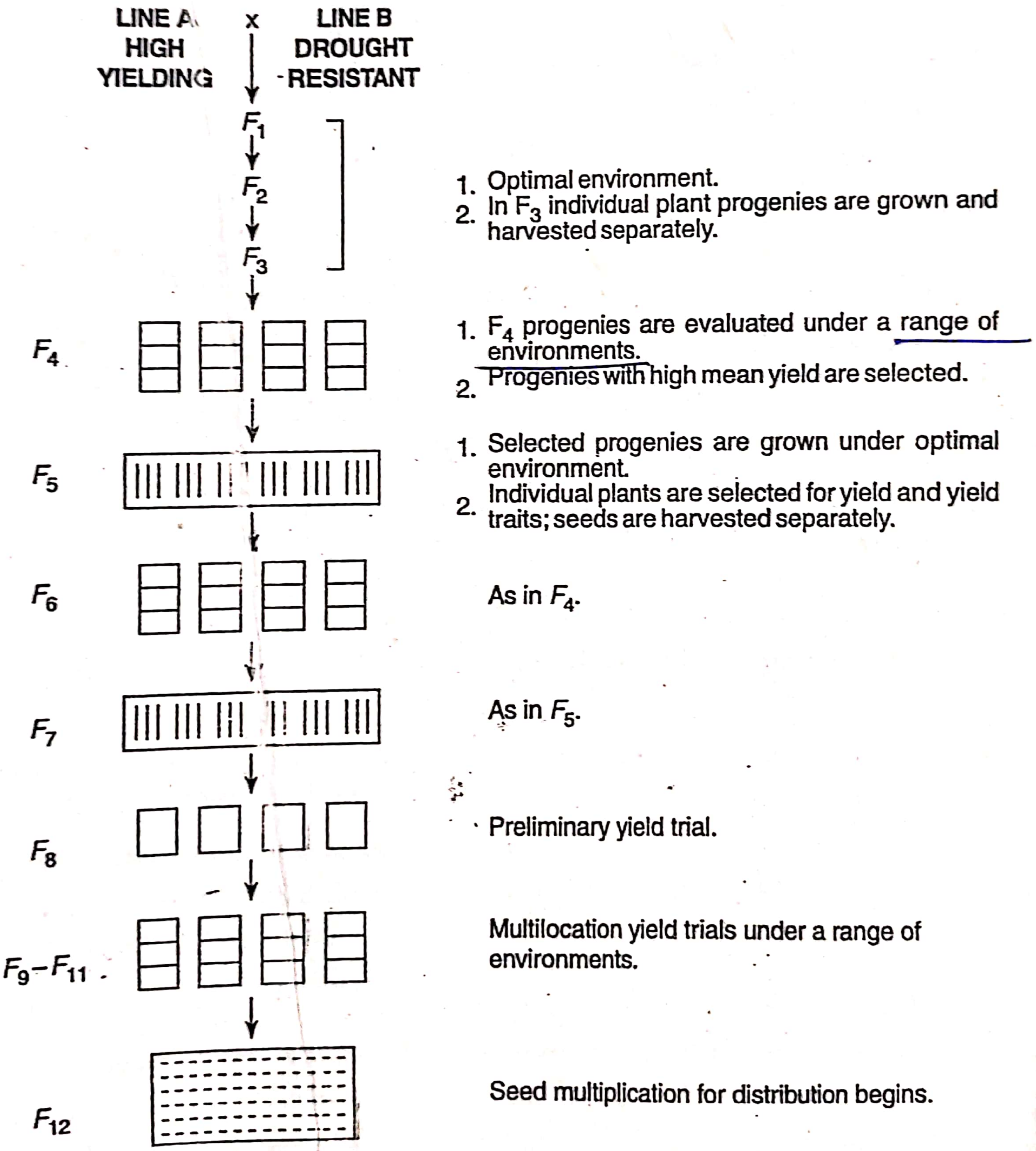

Fig. 24.3. A breeding approach to develop varieties suitable for a range of environments.

suffers from several more limitations, *e.g.*, reduced effectiveness of selection, need for extreme care in field preparation, etc.

24.14.2. Adaptation to A Variable Environment

This approach attempts to combine high yield potential with drought resistance. The selection programmes are based mainly on yield and yield components. The yield stability of selected lines is evaluated under several environments ranging from stress to nonstress conditions. There is some evidence that it should be possible to combine very high yield with wide adaptability. According to one scheme individual plant progenies are evaluated under a range of optimal and moisture stressed environments, and selection among progenies is based on their average performance over all the test environments. The selected progenies are grown under optimal conditions and within progeny selections are carried out for yield and yield attributes (Fig. 24.3). The materials evolved from breeding environments are finally evaluated under a range of environments and those showing high mean performance with high stability may be released for cultivation.

24.14.3. Combining Selection for Drought Resistance Traits and High Yield Potential

In this approach selection for yield potential and drought resistance traits, *e.g.*, cuticular wax in Fig. 24.4, are integrated into a single scheme. The F_1 is advanced to F_4 under optimal moisture. In F_4, a large number of individual plants are selected and harvested separately. In F_5, individual plant progenies are grown under moisture stress, and progenies are evaluated for cuticular wax only; superior progenies are harvested separately.

The selected progenies are grown under optimal moisture in F_6, and selection is based on yield, yield attributes and quality traits. It may often be necessary to repeat the operations of F_5 and F_6 one or more times to identify desirable progenies. The selected progenies would be subjected to multilocation trials before release for commercial cultivation.

24.14.3.1. A Dyanamic Breeding Scheme. A comprehensive scheme for breeding for drought resistance and high yield potential has been proposed, which takes advantage of the recurrent selection procedure (Paroda, 1986). The salient features of this scheme are outlined below.

Step I

1. Multilocation evaluation under stress conditions is done to identify stable and drought resistant lines.
2. Crosses are made between drought resistant lines and agronomically superior cultivars with a view to combine high yield potential with drought resistance.
3. F_1, F_2 and F_3 are grown under nonstress conditions. In F_3, individual plant progenies are evaluated for yield attributes and selection is based on these data.
4. In F_4, selected progenies are evaluated under stress (to identify stress resistant lines) as well as nonstress conditions (for seed multiplication). Stress resistant progenies are identified and grown in F_5.

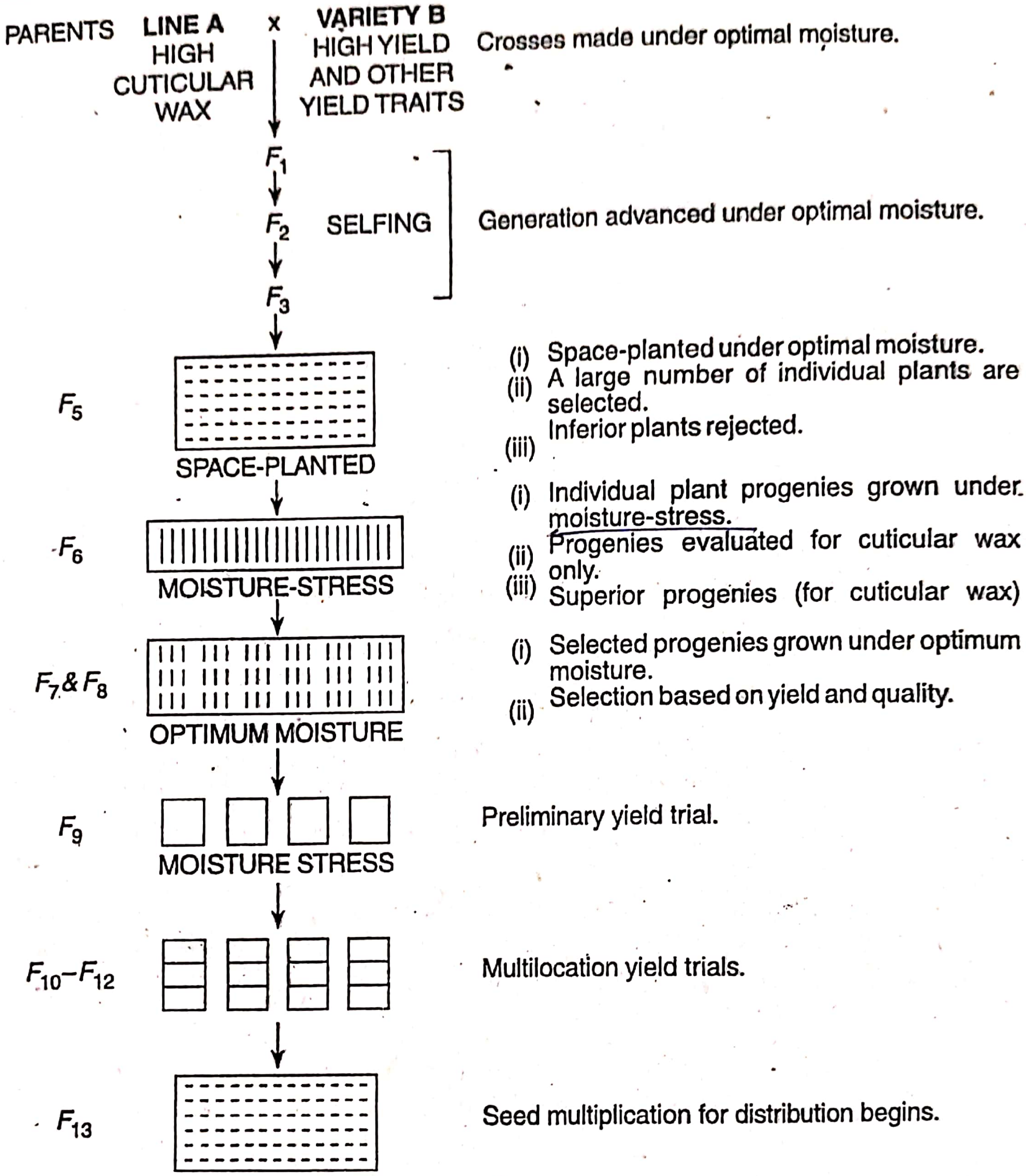

Fig. 24.4. A breeding approach for drought resistance based on a combined use of optimum and moisture stress environments.

5. In F_5, selected progenies are handled as in F_4.
6. Ultimately, the selected lines are subjected to multilocation tests and released for commercial cultivation.

Step II

1. Selected F_5 lines are crossed to identified sources for desired drought resistance traits.
2. Procedures listed under items 3-6 are executed.

It has also been suggested that multiple cross approach may be used with a view to assemble different desired attributes simultaneously after the source genotypes have been identified. Breeding methods, such as, recurrent selection could be used for this purpose (Paroda 1986). It may be pointed out that virtually all the useful drought resistance traits are under polygenic control. Therefore, pedigree method is the most obvious breeding method, unless the drought resistance trait is to be transferred from an agronomically inferior land race (in which case, 1-2 backcrosses with the cultivated variety should be made) or a wild species (where backcross method would be used). Selection for drought resistance traits is ordinarily performed on individual plant progenies; it is rarely done on individual plants. It has, therefore, been suggested to delay the evaluation for stress resistance till F_4 or later generations and carry the segregating generations as bulks in F_2 and F_3 (mass-pedigree method).

24.14.3.2. Barley Breeding at ICARDA, Syria. The barley breeding strategy at ICARDA, Syria is as follows: (1) direct selection for adaptation to the target environment (selection based on yield and other traits), (2) use of locally adapted germplasm, and (3) use of field experimental techniques and designs to minimise environmental variation. The short-term objective of the programme is to isolate superior purelines from the landraces adapted in the region. On the other hand, the long-term objective is to develop mixtures of superior, genetically different purelines selected from the land races so that they are better adapted to the unpredictable environment in the concerned dry regions.

The field technique at ICARDA, Syria consists of (1) the use of large plots, (2) ensuring that there are no missing rows, (3) using a series of well chosen checks at regular intervals, (4) adjusting plot means for the distance from the nearest check and expressing them as per cent of check mean, and (5) using improved statistical designs like generalised lattice and nearest-neighbour analysis (extensively used in Australia). These techniques, if used carefully, restrict the environmental variability at high stress sites at levels comparable with those of high input research stations (Ceccarelli and Grando, 1996, in Belhausen, 1997). The salient features of the breeding method used at ICARDA, Syria are summarized below.

1. Breeding materials (purelines selected from land races, segregating bulks, introductions, etc.) are tested in the target environment using farmer's agronomic practices under rainfed conditions. The material is also evaluated at the main research station under rainfed but moderate input conditions.
2. Grain yield is the major selection criterion, and only the material outyielding the best check is promoted to further testing. [If a rejected line/population possesses some useful traits, it is saved as parental material.] Other selection criteria are, early growth vigour, plant height, tillering, disease resistance, earliness, etc.
3. The breeding material is tested at least for 3 years.
4. The crosses are evaluated as above before individual plant selection begins. If the number of crosses is small, several random families are isolated from each cross and used for evaluation (earliest trial can be planted in F_3). But if the number of crosses is large, the segregating populations are evaluated as bulks (trial can begin in F_3).
5. Individual plant selections are done only in the best crosses. Obviously, the selection can start only in F_5/F_6 after 3 years of evaluation. Thus the breeding method is a type of bulk-pedigree scheme.